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DEVELOPMENT OF HPLC METHOD FOR DETERMINING THE FOREIGN IMPURITIES IN THE SUBSTANCE OF CARBOREN

Natalia L. Bereznyakova [*1], Sergii V. Baiurka [2], Andrii V. Berezniakov [3], Vitaliy D. Yaremenko [1] and Larisa A. Bobritskaya [4]

Department of Medicinal Chemistry [1], Department of Drug and Analytical Toxicology [2], Department of Clinical Pharmacology [3], Department of Industrial Technology [4], National University of Pharmacy, Kharkiv, Ukraine.

Keywords:

Carboren, Parent substance, Organic impurities, HPLC

Correspondence to Author:
Natalia L. Bereznyakova

Doctor of Pharmacy and Professor, Department of Medicinal Chemistry, National University of Pharmacy, Kharkiv, Ukraine.

E-mail: nataliberezniakova@gmail.com

ABSTRACT: A High-Performance Liquid Chromatography (HPLC) technique has been developed for the determining of foreign impurities in the parent substance of carboren. We have proposed the most sensitive, selective and also the universal HPLC method to be used, which would allow to conduct the qualitative and quantitative analysis under the same conditions so that to determine foreign impurities in the substance. Reliable separation of carboren and impurities was performed on a liquid chromatography equipped with a spectrophotometric detector and an analytical stainless steel column (250 × 4, 6 mm) of size, filled with C18 sorbent (5m km), in gradient elution mode. The detection of limits for carboren and identified of potential organic impurities were determined. It was found that the content of any other individual impurity did not exceed 0.1%, the total content of impurities did not exceed 0.5%. In the samples, impurities A, B and C were detected with a relative retention time of 0.35; 0.42 and 0.30. The results were repeatedly reproduced in model mixtures, parent substance samples.

INTRODUCTION: Carboren- 6- hydroxyl - N-(4-methoxyphenyl)-4-oxo-1,2-dihydro-4H-pyrrolo-[3, 2,1-*ij*]-quinoline-5-carboxamide is a synthetic organic compound synthesized in the National University of Pharmacy under the supervision of Professor I. V. Ukrainets [1]. Carboren displayes signified diuretic and potential antihypertensive effect in the absence of side effects typical for this group of drugs, such as water-electrolyte balance disturbance, kidneys excretory function *etc.* [2]. While pharmacological studies, it has been established, that this substance is normalizing parameters of a renal homeostasis, restores kidneys' function and shows diuretic activity.

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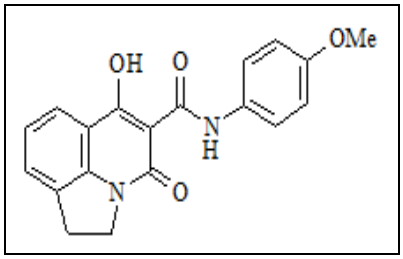
In comparison of the hydrochlorothiazide, it significantly exceeds it on in the strength of the action [3, 4]. Due to its specific physiological activity in a body, carboren is supposed to be applied as a substance for pharmaceutical use (further the substance) in the production of non-sterile medicines in the solid dosage forms as an active substance. The purpose of this study was to develop a methodology for determining impurities in a substance using HPLC.

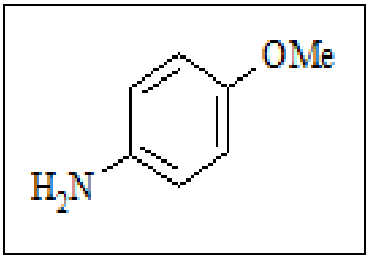
MATERIALS AND METHODS:

Experimental Part: The experimental and pilot industrial series of substances were used in order to develop an analytical technique and to determine the limiting values of foreign impurities content. Conditions for the analysis (sample preparation, chromatographic system's parameters) were selected for selective, with acceptable sensitivity, limiting determination of potential impurities obtained while synthesis and the substance's storage.

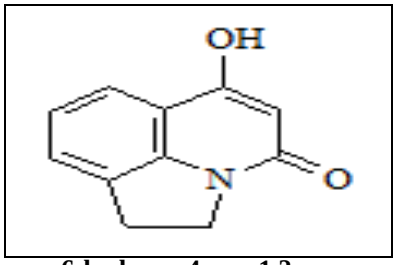
When developing the procedure for the foreign impurities' determination, the starting, intermediate products of the synthesis and a product caused by degradation of the caboren decarboxylation: 4-methoxyaniline (impurity A), 6-hydroxy-4-oxo-1,2-dihydro-1H-pyrro-lo[3,2,1-*ij*]quinoline (impurity B), 6-hydroxy-5-ethoxycarbonyl-4-oxo-1, 2 -dihydro-1 H-pyrrolo[3,2,1-ij] quinoline (impurity C) were applied as tracking substances **Fig. 1**.



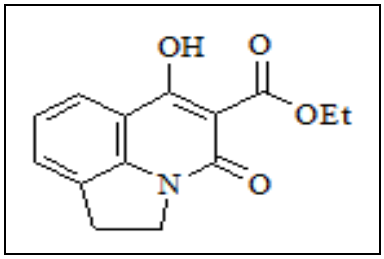
Caboren (Substance)



4-methoxyaniline (impurity A)



6-hydroxy-4-oxo-1,2-dihydro-1H-pyrro-lo[3,2,1-*ij*]quinoline (impurity B)



6-hydroxy-5-ethoxycarbonyl-4-oxo-1,2-dihydro-1H-pyrrolo[3, 2,1-*ij*] quinoline (impurity C)

FIG. 1: MOLECULAR STRUCTURE OF CARBOREN AND IMPURITIES

The study was carried out on a liquid chromato-graph with a UV detector at a detector wave 235 nm in length, on the analytical column Agilent Zorbax SB-CN with a length of 250 mm and internal diameter of 4.6 mm with particles of 5 μm in size.

RESULTS AND DISCUSSION: The substance is a crystalline powder of light yellow color, practically insoluble in water and alcohol, slightly soluble in chloroform and sparingly - soluble in methylene chloride with a melting point of about 200 °C [5]. In solutions, the substance is like a substance quite stable to the action of acids, alkalis, oxidants and light, and the high hydrophobicity contributes to its stability when stored.

We have proposed the most sensitive, selective, and also the universal HPLC method to be used, which would allow to conduct the qualitative and quantitative analysis under the same conditions so that to determine foreign impurities in the substance [6]. The estimation of each impurity's content and their total amount in a substance is carried out with the comparison solution prepared by 200-times dilution of the test solution (0.5% of the caboren in the examined solution).

When preparing the mobile phase (a mixture of 0.4% solution of tetrabutylammonium hydrogen sulfate in a phosphate buffer solution with pH = 6.1 and acetonitrile (1:1)), di-calcium hydrogen phosphate, tetrabutyl ammonium hydrogen sulfate, water for chromatography were used. The value of the solution's pH is adjusted to 6.10 ± 0.05 (potentiometrically) with the concentrated phosphoric acid. The rate of flow in the mobile phase is 1.0 ml/min, the temperature of the column thermostat is 30 °C.

In the circumstances, the time of the substance retention was 11.18 ± 0.02 min, of the impurity A - 3.68 ± 0.04 min, of the impurity B - 4.72 ± 0.02 min, of the impurity C - 2.95 ± 0.03 min. The chromatogram of the model mixture of the substance and its impurities (concentration of the substance 0.5 %, impurity A 0.03 %, impurities B and C 0.1 % each of them) has shown in the **Fig. 2**. An analysis of the serial samples of the substance was carried out under the mentioned conditions.

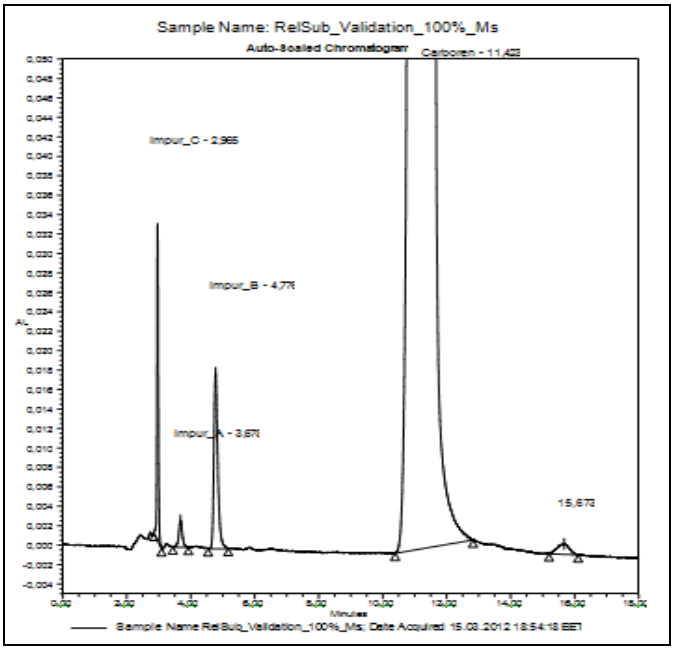


FIG. 2: HPLC-CHROMATOGRAM OF A MODEL SOLUTION OF THE SUBSTANCE WITH THE LIMITS OF IMPURITIES

The model solutions of the substance were prepared as follows: 0.050 g of the preparation was placed into a volumetric flask of 100 ml, dissolved in 80 - 90 ml of acetonitrile, the volume of the solution was completed by acetonitrile to the mark, and mixed.

To prepare a solution of the comparison, a sample of 10.0 mg of 4-methoxyaniline is placed into a 100 ml volumetric flask, dissolved with an ultrasonic bath in 80-90 ml of acetonitrile, the volume of the solution is brought to the mark by acetonitrile and mixed. 1.0 ml of the final solution is placed into a 50 ml volumetric flask, the volume of the solution is adjusted to a mark with acetonitrile and mixed (initial impurity solution). For checking the chromatographic system's suitability, solution of the substance was prepared in a concentration of 0.5 mg/ml and a reference solution (4-methoxy-aniline) in a concentration of 0.1 mg / ml, and then chromatographied under the conditions described above. Suitability of the chromatographic system was evaluated by the efficiency of the chromate-graphic column (not less than 4000 theoretical plates, for the substance peaks), the symmetry factor calculated for the substance peaks (not more than 1.5), the relative standard deviation calculated for the peak areas of the substance in parallel measurements (not more than 1.0%) and relative time of retention (not less than 1.0 relatively to the substance).

On the backgrounds of analysis of 5 samples of the substance, it is possible to make a conclusion about the maximum possible foreign impurities in the substance during the synthesis and storage. As is obvious from the data obtained, the substance can contain predictable impurities B and C, as well as up to 4 unidentified impurities, moreover one of them (with a retention time of about 15.2 min or a relative retention time of about 1.36) is present in almost all samples in different amounts (from 0.03 to 0.23%).

However, in the amount of 0.23%, this impurity was detected in only in one sample obtained at the stage of technology development for the substance obtaining, and subsequently its amount never exceeded 0.1% in all laboratory samples of the substance. The content of any other individual impurity did not exceed 0.1%, the total content of impurities did not exceed 0.5%. In the samples, impurities A, B and C were detected with a relative retention time of 0.35; 0.42 and 0.30 **Table 1**.

TABLE 1: THE CONTENT OF FOREIGN IMPURITIES IN SAMPLES OF THE CARBOREN SUBSTANCE

Sample	Impurity A	Impurity B	Impurity C	Unidentified impurities	Impurities in total
1	undetected	undetected	undetected	0.012 %	0.012 %
2	undetected	0.021 %	0.030 %	0.032 %	0.083 %
3	undetected	undetected	0.021 %	0.028 %	0.049 %
4	undetected	undetected	undetected	0.017 %	0.335 %
				0.065 %	
				0.018 %	
				0.234 %	
5	undetected	undetected	undetected	0.018 %	0.057 %
				0.039 %	

CONCLUSION: Thus, the conditions selected for the analysis of the carboren substance by the HPLC methodique allow to separate industrial impurities, and products of decarboxylation from the number of unidentified impurities. The HPLC methodique provided allows detecting foreign impurities in a

content of not more than 0.1% and not more than 0.5% of all impurities in total. The developed thodique will be included in the quality control methods for this substance.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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