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LABORATORY INVESTIGATION DURING THE CONTROL OF THE PHARMACEUTICAL DRUGS ON THE INDICATOR "RELATED SUBSTANCES" BY THE METHOD OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract. *We provided the laboratory investigation during the control of the pharmaceutical drugs on the indicator "Related substances" by the method of high-performance liquid chromatography (HPLC). It allows us to study the related substances of the pharmaceutical drugs. Also we considered three hypotheses which could affect the final result. Consequently, the sample preparation procedure was found to be an error made by the laboratory personnel.*

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Introduction

During the production of medicines, the substance is subjected to various influences, which may result in decomposition processes or other adverse reactions. Substance and finished pharmaceuticals may contain impurities - breakdown products and processing impurities [1]. The Russia State Pharmacopoeia requires all pharmaceutical substances be subjected to testing for 'Related substances' in order to identify the impurities. All the identifiable and non-identifiable impurities should be identified. There are limits to their content. If deviations from the prescribed limits are found, an investigation into the deviations is required during quality control of the pharmaceutical product. This procedure is an integral part of the pharmaceutical quality system.

The release of pharmaceuticals and substances within the requirements of Federal Law No. 61-FZ of 12 April 2010 "On Circulation of Medicines" implies no other impurities except as naturally occurring in the regular manufacturing process. There also should not be any impurities introduced accidentally, e.g. due to poor cleaning of the processing equipment, the so-called foreign impurities [1, 2].

High-performance liquid chromatography (HPLC) is a method to analyse the presence of any impurities in a pharmaceutical product or substance [3, 4].

The main body

The active pharmaceutical ingredient (API) of a hypolipidemic drug was chosen as the object of study. When testing the investigational pharmaceutical substance for "Related substances", we detected the deviations from the values given in the regulatory documentation. According to the existing procedures in a pharmaceutical company, two challenges should be considered:

1) Determine whether impurities are identifiable or non-identifiable ones.

2) Identify the reason for such deviations.

To solve the first problem, it is necessary to determine which impurities will be related to the test API. The subject belongs to the group of statins. Statins are a group of pharmaceuticals with the primary mechanism of action being the inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA), an enzyme involved in cholesterol synthesis (mainly in the liver). By inhibiting a key step in sterol biosynthesis, statins are the main medicines reducing cholesterol levels and preventing cardiovascular events (5).

The mechanism of action of the medicine under study is determined by inhibition of HMG-CoA reductase, an enzyme limiting the cholesterol (cholesterol) synthesis stage, which reduces the production of mevalonic acid from HMG-CoA. Inhibition of HMG-CoA reductase leads to an increase of low density lipoprotein (LDL) receptors on hepatocyte membranes, stimulation of LDL catabolism, and a decrease of high-sensitivity C-reactive protein. In addition to its hypolipidemic action, rosuvastatin has pleiotropic properties, including inhibition of platelet aggregation, anticoagulant effect, reduction of inflammation in atherosclerotic plaque, and improvement of endothelial function [6].

An analysis of the references [6] shows the following related substances are characteristic of the PI under study:

7-[4-(4-fluorophenyl)-6-isopropyl-2-[N-methyl(N-methylsulfonyl)amino]pyrimidine-5-yl]-(3R,5R)-3,5-dihydroxy-(E)-hept-6-enoic acid or anti-isomer (3R,5R). This substance is a processing impurity.

7-[4-(4-fluorophenyl)-6-isopropyl-2-[N-methyl(N-methylsulfonyl)amino]pyrimidine-5-yl]-3R-dihydroxy-5-oxo-(E)-hept-6-enoic acid or 5-keto acid impurity is a degradation impurity.

6-[(E)-2-[4-(4- fluorophenyl)-6-isopropyl-2-[N-methyl(N-methylsulfonyl)amino]pirimidin-5-yl]-vinyl]-4-hydroxytetrahydro-2H-piran-2-one or lactone is a processing impurity.

The impurity detection tests are conducted by high-performance liquid chromatography. Solutions for each series are prepared for analysis: a solution to check the system suitability and a solution to identify the peaks obtained in the chromatograms.

Two unidentified impurities were detected in the test solution of the first series. The retention time (RT) of the first one is 35 minutes, it is 0.40% mass. The retention time of the second one is 40 minutes, it is 0.37% mass. Tests of the second series revealed similar unidentified impurities: the RT of 35 minutes is 0.42% mass., with an RT of 40 minutes it is 0.40% mass.

The normative documents stipulate the presence of unidentified impurity should not be more than 0.2% mass. Thus, the tests for "Related substances" revealed extraneous peaks which were not present in the tests of similar series before. Their appearance in the test results is an exception and should be investigated according to an appropriate procedure. The occurrence of unidentified impurities in the chromatograms is shown in Fig. 1, 2.

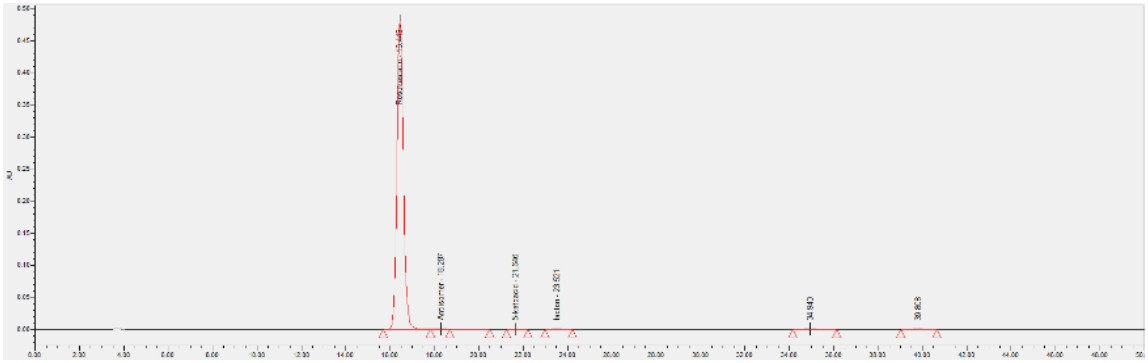

Fig. 1. Chromatogram of test solution in series 1

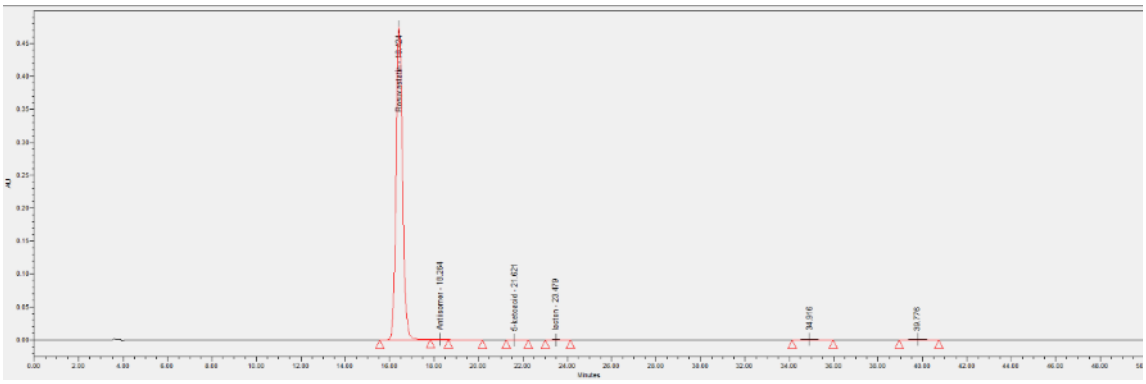

Fig. 2. Chromatogram of test solution in series 2

We conducted the system suitability test [7] as an additional study. The chromatogram for checking the suitability of the system is shown in Fig. 3; the chromatogram of the solution for identifying impurities is shown in Fig. 4. There are no peaks on the chromatograms found on the chromatograms of the test series. Therefore, they are not systematic.

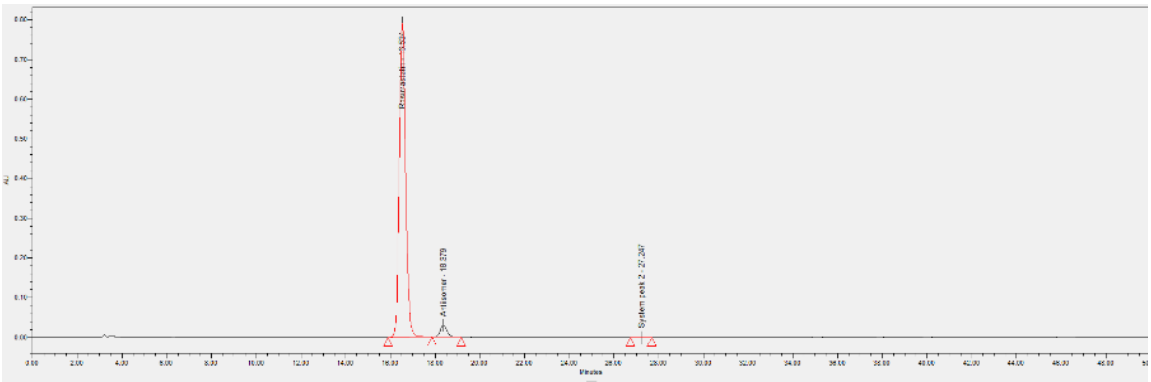

Fig. 3. Chromatogram system suitability test

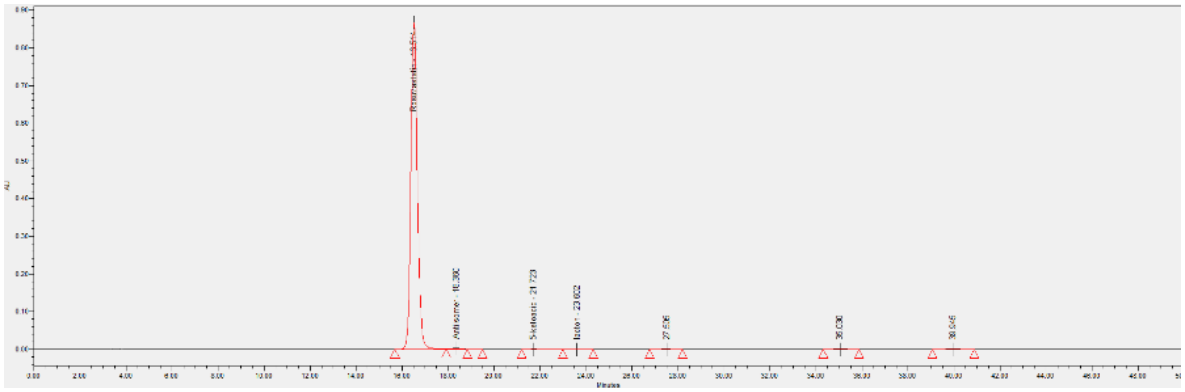

Fig. 4. Chromatogram of the solution for the impurities identification

The analysis shows the peaks obtained are not a product of the API decomposition. It can be assumed the impurities occur during sample preparation.

A few hypotheses have been put forward for the appearance of unidentifiable impurities as a result of contamination at the sample preparation stage:

1) Contamination occurs at the filtration stage.

2) The sampling dishes (pipettes) were insufficiently cleaned.

3) During the tests solvent contamination occurred.

To verify the hypotheses put forward, the quality control department conducted an investigation.

To test the first hypothesis, the initial solutions were re-filtered through a filter that was used for all tested solutions. Final solutions are also re-prepared. These peaks did not appear on the chromatograms of the re-prepared solutions, which confirm the purity of the initial test solutions. Contamination probably occurred during the preparation of final solutions.

To test the second hypothesis, we conducted an analysis of the sampling utensils. The pipettes used were different for the different dosages of the test substance. Accordingly, if one pipette had been used, the contamination would have been of the same series. Consequently, the effect of pipettes has not been confirmed.

The third hypothesis is probably the using of contaminated solvent. Assumed the solvent poured into the plastic measuring cup to prepare the test solution might have been contaminated. The chromatograms of the blank, the standard solutions, and the re-filtered test solutions show no peaks related to unidentified impurities. It confirms the purity of the original solvent. It also confirms the purity of the original test solutions and the contamination of the final solutions during the preparation process. When interviewing the personnel, it was found out different cups were used to add the solvent into the initial and final test solutions. It is noticed the measuring cup is signed with a marker which does not wash off very well. Since the solvent includes methanol and acetonitrile, the marker may have dissolved well. The marker could have entered the solution by pooling at the spout of the cup when the solvent hit the outer walls, which often happens when solvent is added to the flask. To confirm this assumption, the solvent with which the marker was washed off the cup was stabbed. In a dishwasher with a measuring flask holder, cups fall off the holders and remain unwashed. Thus, as a result of the laboratory investigation on the indicator "Related substances" of the API, personnel error in the

preparation of test solutions has been identified. According to the Pharmaceutical Quality System it is necessary to re-train the personnel on the procedure for preparation of test solutions.

Conclusions

Thus, we detected abnormalities of unidentifiable impurities during an API investigation of a pharmaceutical product with a hypolipidemic effect. Three hypotheses were put forward and tested in the course of the investigation.

The most probable explanation is a poorly washed plastic cup in which solvent was poured. These particular cups were then used to prepare the test solutions.

Thus, the identified deviation during the tests according to the indicator "Related substances" is the personnel error during the preparation of the test solution. To prevent this deviation, it is necessary to re-train the laboratory personnel to perform this procedure.

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