OPTIMIZATION OF THERAPEUTIC DRUG MONITORING FOR PATIENTS WITH HEART TRANSPLANTATION

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Everolimus is an immunosuppressant widely used in clinical practice for the prevention of transplant rejection in adult recipients. The narrow therapeutic range of concentrations and the high variability of the pharmacokinetic parameters of the drug require constant monitoring its blood concentration.

The purpose of the study was to develop reliable, selective, sensitive and reproducible technique for determining everolimus in human whole blood by high performance liquid chromatography (HPLC) method with mass spectrometric detection.

Materials and methods. The study was performed using a high performance liquid chromatograph "LC 1260 Infinity" (Agilent, USA) with mass spectrometric detector "TripleQuard 6460" and the ionization system "Agilen Jet Stream – electrospray" using pharmacological substances "Everolimus" and "Everolimus–d4".Calibration and control samples at 1; 3; 5; 7; 9; 11; 13; 15 ng/ml were prepared from whole blood of healthy donors by adding equal volumes of concentrated solutions of everolimus in methanol and Everolimus–d4 as internal standard, to the intact blood.

Results. At the stage of development of technique the sample preparation and implementation of the study protocol was picked up.

Chromatographic separation: the amount of input — 5 mcl; mobile phase: A — 100 mM ammonium formate solution in water containing 0.1% formic acid; B — 100 mM solution of ammonium formate in methanol containing 0.1% formic acid; isocratic — elution mode; flow rate — 0.4 ml/min.

Ass spectrometry Settings: Scan mode — MRM (monitoring given ion reactions), MRM transition"everolimus" — (975.6 - 908.5); MRM transition"everolimus–d4" — (979.6- 912.5).

Data processing: software — Agilent Technologies Mass Hunter B 07.00.

Validation of analytical methods is performed. To evaluate the selectivity of the method we analyzed intact blood sample not containing the analyte of 6 different sources. Sample preparation and sample analysis were performed in the conditions described above.

The inspection results are satisfactory, as the mass chromatograms had no peaks at specified crossings masses with signal/noise ratio greater than 3:1.

Evaluation of reproducibility is made basing on the results of model blood samples analysis at three concentration levels of 10 repetitions (reps required - not less than five). The coefficient of variation (RSD) for the lower limit concentration was — 9.2%; RSD for the average level of concentration was — 3.7%; RSD for a high level of concentration — 2.1%.

The sensitivity of the method was 0.3 ng/mL. The linear range of the method was 1–15 ng/mL, MSE> 0.999. The lower limit of detection — 1 ng/ml. Reproducibility, precision and accuracy is achieved over the entire range of concentrations. Concentration determination method is the method of internal standard.

The results of checking the correctness of the results have been declared admissible, since the measurement error was less than 15% and for the lower limit of quantification was not higher than 20%.

Thus, the characteristics of the technique correspond to the eligibility criteria of the validated bioanalytical method.

This technique has been successfully applied for the determination of everolimus in 50 whole blood samples from 10 patients.

References

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