

# VALIDATION OF SIMULTANEOUS ASSAY OF CLOZAPINE AND ITS MAIN METABOLITES IN BLOOD SERUM USING HPLC-MS/MS METHOD

*Article history:*  
Submitted 20 June 2019  
Accepted 31 July 2019

Olga L. Romanova<sup>1,2\*</sup>, Dmitriy V. Sundukov<sup>1</sup>,  
Arkadiy M. Golubev<sup>1,2</sup>, Mikhail L. Blagonravov<sup>3</sup>

<sup>1</sup> RUDN University, Department of Forensic Medicine, Moscow, Russia

<sup>2</sup> Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russia

<sup>3</sup> RUDN University, V.A. Frolov Department of General Pathology and Pathological Physiology, Moscow, Russia

\*Corresponding Author: [olgpharm@yandex.ru](mailto:olgpharm@yandex.ru)

## INTRODUCTION

Clozapine is an atypical antipsychotic drug. It is frequently used in clinical practice. The number of criminal clozapine poisonings remains high. Clozapine is metabolized in the liver. Its main metabolites are norclozapine and clozapine-N-oxide [1]. According to the literature these metabolites have their own toxic effect [2]. However there is no validated method of simultaneous assay of the substances.

### *The aim of the study*

is to carry out the validation of simultaneous assay of clozapine and its main metabolites — norclozapine and clozapine-N-oxide — in blood serum using HPLC-MS/MS method. We represented calibration curves for clozapine and its main metabolites. We assessed the following parameters: selectivity, precision, accuracy, matrix effect, lower limit of quantification [3].

## MATERIALS AND METHODS

The validation was carried out using chromatograph Agilent Technologies 430 Triple Quad LC/MS (Germany). We used PC programme Agilent Mass Hunter Workstation for series tripple Quadrupole vers. B06.00 build 6.0.6.25.4sp4 to represent chromatograms and Agilent Mass Hunter Quantitive Analysis vers. B 07.00 build 7.0.457.0 to proceed them.

## RESULTS

**Calibration curve.** We used six levels of concentration for clozapine. The range of concentrations was from 0,125 to 5  $\mu$ /ml. Relative standard deviation (RSD) for used clozapine levels did not exceed 15%, accuracy was between 88,15% and 109,51%. We used

seven levels of concentration for norclozapine. The range of concentration was from 0,0075 до 7,5  $\mu$ /ml. Relative standard deviation (RSD) for used norclozapine levels did not exceed 15%, accuracy was between 80,13% (concentration level 1) and 106,51%. We used five levels of concentration for clozapine-N-oxide. The range of concentration was 0,03–3,00  $\mu$ /ml. Relative standard deviation (RSD) for used levels of clozapine-N-oxide did not exceed 15%, accuracy was between 81,20% and 98,30%.

**Precision and accuracy.** Precision and accuracy for QC samples should not exceed 15% except the one for the LLOQ which should not exceed 20%. We assessed the precision and accuracy 3 times in 4 QC samples QC\_1, QC\_2, QC\_3, QC\_4 at the expected range of concentrations. All the figures we obtained met the criteria.

**Lower limit of quantification.** The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision. The LLOQ is considered being the lowest calibration standard. The lower limit of quantification we obtained for clozapine was 0,05  $\mu$ /ml. The *signal/noise ratio* (S/N) was 0,12. The lower limit of quantification we obtained for norclozapine was 0,0025  $\mu$ /ml; S/N was 0,14. The lower limit of quantification we obtained for clozapine-N-oxide was 0,001  $\mu$ /ml; S/N was 0,10.

**Recovery.** The recovery rate for clozapine was measured at concentrations of 5  $\mu$ /ml and 0,25  $\mu$ /ml, the recovery rate for norclozapine was measured at concentrations of 0,5  $\mu$ /ml and 0,1  $\mu$ /ml, clozapine-N-oxide — 1  $\mu$ /ml and 0,03  $\mu$ /ml. The recovery rate for each analyte and inner standard (IS) — amitriptyline — was estimated separately. The recovery rate for clozapine was 86,6%, the recovery rate for IS exceeded 99%, CV<15%. The recovery rate for norclozapine was 63% the recovery rate for IS exceeded 93%, CV<15%. The recovery rate for clozapine-N-oxide was 84% the recovery rate for IS exceeded 97%, CV<15%.

## CONCLUSION

The validated method can be used for simultaneous assay of clozapine, norclozapine and clozapine-N-oxide in blood serum.