

## SUMMARY

The aim of this work was to verify the correlation at determination of methotrexate by high performance liquid chromatography and immunochemically determination of whole methotrexate. Methotrexate belongs to the chemotherapeutic agent commonly used in the treatment of acute lymphoblastic leukemia. Methotrexate was determined chromatographically with UV detection at 303 nm after deproteinization with trichloroacetic acid. Fluorescence polarization immunoassays of methotrexate was measured on TDx FLx analyzer. The data obtained were analyzed utilizing the PrismGraph Pad 5.0 software. The methotrexate measurements were evaluated employing nonparametric paired t-test (p-value <0,05). Our data indicate good correlation between methotrexate levels > 1  $\mu\text{mol/l}$  determined by high performance liquid chromatography and fluorescence polarization immunoassays. While the concentration of methotrexate < 1  $\mu\text{mol/l}$  measured by fluorescence polarization immunoassays were overestimated. This could be done because of cross reactivity with metabolites 7-hydroxymethotrexate and 2,4-diamino-N<sup>10</sup>-methylptericoic acid. These metabolites could influence the determination of methotrexate, because of the close structural similarities.