

***Feasibility of Quantitative Determination of Methotrexate with Surface-enhanced Raman Spectroscopy and Multivariate Calibration Analysis***

Stefano Fornasaro<sup>1</sup>, Silvia Dalla Marta<sup>1</sup>, Alois Bonifacio<sup>1</sup>, Marco Rabusin<sup>2</sup> and Valter Sergo<sup>1</sup>

<sup>1</sup>Department of Engineering and Architecture, University of Trieste, Trieste; Italy

<sup>2</sup>Institute for Maternal and Child Health (I.R.C.C.S.) Burlo Garofolo, UO Pediatric Hemato-Oncology, Trieste; Italy

Abstract

Surface-enhanced Raman spectroscopy (SERS) coupled with multivariate statistical analysis is fast becoming a promising analytical tool for therapeutic drug monitoring. In comparison with standard techniques such as HPLC, it has the remarkable advantages of rapidity, simplicity, low cost, and no need for sample pretreatment.

Methotrexate (MTX) is a folate antagonist widely used for treatment of various neoplastic diseases in children. However, there is still a need for improving the accuracy of the procedures aimed at therapeutic drug monitoring of MTX.

This study investigates the feasibility of using SERS coupled with partial least-squares (PLS) multivariate calibration for the quantitative determination of MTX.

A complete methodology was implemented for developing the proposed method, including experimental design, data preprocessing, outlier detection, and stability selection. The influence of the preprocessing methods on the prediction speed, robustness and accuracy performance was compared.

The best PLS model was obtained with seven latent variables in the range from 0.1 to 10  $\mu\text{M}$  of MTX, providing a root mean square error of prediction (RMSEP) of 0.74  $\mu\text{M}$ .

Experimental results showed that the combination of SERS with multivariate calibration can provide quite precise concentration predictions for MTX in a clinically relevant concentration range with average relative prediction error of 2%.