OPTIMIZATION OF MOLECULARLY IMPRINTED SOLID-PHASE EXTRACTION BY MEANS OF EXPERIMENTAL DESIGN PRIOR TO THE DETERMINATION OF METHOTREXATE IN HUMAN PLASMA

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A recent trend in bioanalysis is the introduction of molecularly imprinted solid-phase extraction (MISPE) due to their tailor-made selectivity for the determination of traces of analytes in complex matrices by liquid chromatography (LC) [1].
In order to selectively extract methotrexate (MTX), a powerful anti-cancer agent, from plasma samples, molecularly imprinted polymers (MIPs) were prepared against MTX by using two structural analogues, trimethoprim and *N*-*Z*-L-glutamic acid, as templates [2-3]. Non-imprinted polymers (NIPs) were also synthesized without template. These sorbents, packed in disposable extraction cartridges (DECs), were then evaluated for at-line solid phase extraction (SPE) by means of a sample processor (ASPEC system) and coupled directly to LC. The separation was performed on octadecyl silica stationary phase with a mixture of 10 mM phosphate buffer (pH 2.5) and methanol (77:23; v/v) as mobile phase. MTX was monitored photometrically at 307 nm.
The SPE conditions were studied by means of experimental design in order to investigate the retention selectivity and try to explain the retention mechanisms of MTX on the MIPs. The MISPE selectivity was exploited in the washing step to transform non-selective interactions into selective interactions.

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