

## **DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) IN HUMAN URINE BY ONLINE MISPE-LC-UV**

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Nonsteroidal anti-inflammatory drugs (NSAID) have been widely used due to their analgesic, antipyretic and anti-inflammatory activities. An important step in a bioanalytical method is the sample preparation. Solid phase extraction (SPE) is a technique widely used for extraction of analytes in biological fluids. Nowadays, molecularly imprinted polymers (MIP) have attracted attention because due to their higher selectivity than typical SPE phases. Several methods have been developed for NSAID in body fluids, but most of them have many extraction steps. The use of online MIP extraction is an attractive alternative because it lowers the total analysis time, uses low amount of sample and can be automated. The aim of this study is the development of a simple and selective method for direct analysis of NSAID in human urine, requiring a minimal sample pretreatment. Ibuprofen-imprinted amino-functionalized silica gel sorbent was synthesized and the template was removed by reflux. For comparison, non-imprinted polymer (NIP) was prepared under identical procedure, but without the template. LC analysis was performed using a Shimadzu 20A HPLC instrument equipped with a six-port valve connected to a MIP extraction pre-column (20 mm x 4.6 mm) and a NST C18 (150 mm x 2.1 mm x 5 µm) analytical column in order to perform column switching online sample separation. The mobile phase was MeOH:H<sub>2</sub>O (70:30, v/v with acetic acid 0.1%) at a flow rate of 0.2 mL min<sup>-1</sup>. UV detection was performed at 220 nm. The total analysis time was 22 minutes under isocratic elution condition. After optimization of the extraction process and chromatographic separation, the method was validated for ketoprofen, naproxen, flurbiprofen and ibuprofen using fenoprofen as IS. The linear range from 0.5-15 µg mL<sup>-1</sup> with r<sup>2</sup> higher than 0.997, the inter-day accuracy and precision was lower than 13 % and the relative recovery was around 95-105 %. The robustness was evaluated by Youden test. The developed method was used for analysis of NSAID in human urine in free and deconjugated forms.

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