Novel Microextraction Techniques for Bioanalysis of Neurotransmitters and Biomarkers in Biological Fluids

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Abstract

Sample preparation (sample pre-treatment) is the initial step and an essential part in bioanalysis procedure. The main role of sample preparation is to extract and transfer the analyte(s) of interest from a complex matrix to a purified media such as a pure solvent for analysis and quantification. Biological fluids are complex and contain, in addition to the target analyte(s), many different unwanted compounds from salts to proteins. Thus, the analysis of these samples requires an effective sample preparation method prior to the liquid chromatography-mass spectrometry (LC/MS) and gas chromatography-mass spectrometry (GC/MS) assays. The aim of the present work was to evaluate micro-extraction by packed sorbent (MEPS) and develop new sample preparation techniques for the extraction of neurotransmitters and biomarkers from biological samples. Moreover, two new sample preparation techniques were developed. The first developed technique is molecular imprinting on polysulfone membrane (MIPM), and the second one is molecularly imprinted polymer in tablet form (MIPT).

MEPS is a well-known sample preparation technique that can be used online with analytical instruments without any modifications. In this thesis, MEPS was used online with liquid chromatography-tandem mass spectrometry (LC/MS/MS) for the quantification of dopamine and serotonin in human urine samples (Study I) and with GC/MS for the analysis of methadone in humane urine (Study II). Polystyrene polymer and silica-C8 were used as sorbent in Study I and Study II, respectively. In both studies, small sample volumes (50 µL) were used and full method validation was performed. In both studies (I and II), MEPS enhanced the limit of detection (LOD) and reduced the extraction time compared to the previously published methods. In Study I, the mean accuracies of quality control (QC) samples of dopamine and serotonin were 99–101% while the precision values (RSD) were 6–11%. In Study II, the accuracy values of methadone were between 97 and 107% while the precisions (RSD) were between 11 and 15%.

MIP-sol-gel on polysulfone membrane (MIPM) was developed and used in combination with MEPS for the extraction of hippuric acid (HA) in plasma and urine samples (Study III). A good selectivity was obtained using plasma and urine samples. The precision of QC samples in plasma and urine samples were 2.2–4.8% and 1.1–6.7%, respectively. The method recovery was above 90%.

In Study IV, a new technique was developed using a tablet form of molecularly imprinted sol-gel (MIPT) for the extraction of methadone from human plasma samples. Methadone-d9 was selected as the template for accurate recovery, and 3-(propyl methacrylate) trimethoxysilane (3PMTMOS) was used as a precursor. The extraction recovery was higher than 80%. The LOD and LLOQ were 1.0 and 5.0 ng mL^{-1}, respectively. The validation showed good selectivity, accuracy and precision.

In Study I, III and IV, LC/MS/MS system was used while GC/MS was used in Study II for the separation and detection of target analytes.

There will always be a high demand for rapid, selective, reliable and sensitive techniques for sample preparation. It is convenient to use molecular dynamics simulations as a theoretical tool for the optimization of molecularly imprinted system. Suitable monomers and cross-linkers are crucial to synthesize the new membrane and tablet molecular imprinted polymer platforms for all kinds of molecules.

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