VALIDATION RESULTS OF AN LC-MS/MS METHOD FOR THE DETERMINATION OF PFAS IN HUMAN PLASMA SAMPLES

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INTRODUCTION: Per- and polyfluoroalkyl substances (PFAS) are widely used in industrial processes and consumer products. Due to their persistence in the environment and bioaccumulation in the human body, these compounds have been associated with adverse health effects. PFOA, PFOS, and PFHxS are among the most studied PFAS. The sample typically used for their measurement is plasma. Despite the relevance of this topic, there is a lack of biomonitoring studies on human exposure to these substances in Brazil. **OBJECTIVE:** To validate a method for the determination of PFOA, PFOS, and PFHxS in human plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS). **MATERIALS AND METHODS:** Blood samples were collected from volunteers by phlebotomy or fingerpricks to EDTA-containing tubes. Aliquots of 100 µL of plasma were prepared using solid-phase extraction (SPE) with OASIS WAX L cartridges. The chromatographic separation was carried out on an Acquity UPLC HSS T3 reversed-phase column. The mobile phases consisted of an ultrapure water/methanol mixture (95:5) with 2 mM ammonium acetate (Phase A) and methanol with 2 mM ammonium acetate (Phase B). The assay was validated following standard bioanalytical guidelines. **RESULTS AND CONCLUSION:** The method was linear over a calibration curve of 0.2–20 ng/mL. The intra-assay precisions ranged from 3.45 to 5.10%, 3.37 to 6.65%, and 3.41 to 8.12% for PFHxS, PFOA and PFOS, respectively. The inter-assay precisions were 1.06–3.91% for PFHxS, 2.31–4.34% for PFOA, and 0.51–3.83% for PFOS. As for accuracy, the results were 102.6–106.2% for PFHxS, 99.1–102.8% for PFOA, and 97–102.4% for PFOS. Matrix effects ranged from -7.35% to -0.82%. No carryover was observed. Selectivity was confirmed by analyzing a solution of common drugs. Additionally, the PFAS compounds showed stability in autosampler conditions and after four freeze/thaw cycles. The method was applied to 22 paired samples of venous and capillary plasma. PFOS was found in concentrations between 1.10 and 14.2 ng/mL. PFOS quantifications in venous and capillary plasma were highly correlated *(r*=0.951). The stability of PFOS at room temperature was confirmed by analyzing triplicate venous plasma samples from the same volunteer on days 0, 1, 3, 7, and 10 post-collection. The method presented adequate performance for biomonitoring studies.

Keywords: PFAS; LC-MS/MS; SPE; plasma samples; analytical validation.

Financial Support: CNPq, CAPES, and FAPERGS.