Application of Sub/supercritical Fluid Extraction as a New Sample-Preparation Procedure for Isolation and Identification of a Pharmaceutical From Biological Fluids: Application to Disposition Kinetics

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Since its commercial development in the early 1990s, supercritical fluid extraction (SFE) has attracted considerable attention as a sample-preparation procedure. However, other different sample preparation procedures, including precipitation, liquid- and/or solid-phase extraction, still remain in popular use in biological fluids. In this investigation, SFE was introduced to isolate and identify orbifloxacin (OBFX), from plasma and milk. Four parameters, including the temperature and the pressure of supercritical fluid, modifier ratios and dynamic extraction time, were evaluated and optimized to obtain the best yield of the analyte from the biological fluids. Determinations of the OBFX in the extracts were carried out using HPLC-FLD. The optimum conditions of the extraction process that yielded the maximum extraction efficiencies (recovery %) of the analyte were 150°C vs. 60°C, 250 kg/cm², 30% vs 35% methanol, and 40 min vs. 20 min, for plasma and milk, respectively. Good linearity (at least $r^2 \geq 0.999$) of the calibration curves was obtained over the range from 0.2 to 0.01 µg mL\textsuperscript{-1}. The method gave quite good extraction efficiency (recovery rate: 74.2–127.73%) and precision (RSDs: 1.64–20%). The instrumental LOD and LOQ values were 0.004 µg mL\textsuperscript{-1} vs. 0.01 µg mL\textsuperscript{-1}, or 0.006 µg mL\textsuperscript{-1} vs. 0.02 µg mL\textsuperscript{-1}, for plasma and milk, respectively. The method was successfully applied to estimate the pharmacokinetic variables in lactating does following IV and IM administration of OBFX at a dose rate of 2.5 mg kg\textsuperscript{-1} bwt. To the best of our knowledge, this is the first time SFE has been applied to isolate an antimicrobial agent from biological fluids. This method is promising for clinical applications and for pharmacokinetic studies of various pharmaceuticals in biological fluids.