

**DEVELOPMENT OF AN LC-MS/MS METHOD FOR THE DETECTION OF
PREDNISOLONE DOPING USING HUMAN FINGERNAILS**

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Sports doping detection mainly relies on urine and blood samples. Nails are less commonly used, even though they can provide long-term drug use, due to challenges in preparation and pulverization. This study developed a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method to detect prednisolone in human fingernails. Nails from volunteers not administered with prednisolone were ethically collected, cleaned, dried, and pulverized for analysis. The extraction of prednisolone was optimized for solvent type, extraction frequency, and shaking rate. The optimized sample preparation was followed by cleaning of Nail clippings by vortexing at 2200 rpm using ultrapure water at 50 °C for 75 secs, drying at 50 °C for 80 mins, cutting into small pieces, and subjected to methanol extraction by shaking for 18 hrs at 300 rpm. LC-MS/MS analysis used a biphenyl column (100 mm × 2.1 mm, 2.7 µm) with a binary gradient of acidified (0.1% formic acid) acetonitrile and an aqueous phase to identify prednisolone. The run time for the LC-MS/MS method was 7.50 minutes. The retention time of prednisolone was 2.18 minutes. The method was validated according to International Council for Harmonization (ICH) guidelines, showing strong linearity ($R^2 > 0.99$) and high specificity with no carryover. Within-run accuracy and precision were $\pm 3.24\%$ and 3.76% coefficient of variation (CV), respectively, while between-run accuracy and precision were $\pm 4.18\%$ and 3.5% CV, respectively. LOD and LOQ were 0.0003 mg/L and 0.00154 mg/L, respectively. Using a cost-effective pulverization setup, the validated The LC-MS/MS method effectively detected prednisolone in fingernails. Further studies with prednisolone-doped nails are needed to advance nail-based doping detection in sports and forensics.

Keywords: Doping, LC-MS/MS, Nail extraction, Prednisolone