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Determination of cannabinoids in 50 microliters whole blood samples by online extraction using turbulent flow chromatography and LC-HRAM-Orbitrap-MS.

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Introduction and Aim: The analysis of cannabinoids in whole blood is usually done by traditional MS techniques, after offline cleanup or derivatization steps which can be lengthy, laborious, and expensive. We present a method for the determination of THC, CBD, CBN, OH-THC, THC-COOH in 50 µL whole blood samples by online extraction using turbulent flow chromatography and LC-HRAM-Orbitrap-MS.

Materials & Methods: After the addition of deuterated ISs and a simple protein precipitation step, an online extraction of sample supernatants using turbulent flow chromatography (TurboFlow - Thermo Scientific) was carried out. Analytes were separated on a C18 analytical column and detected by LC-HRAM-Orbitrap-MS using a Thermo Scientific Q Exactive Focus MS system. MS detection was performed in polarity switching and SIM modes using five specific acquisition windows, at a resolution of 70,000 (FWHM).

Results & Discussion: Total run time was about 10 minutes including pre-analytical steps. LLOQs were 0.5 ng/mL for THC, CBN, and THC-COOH, and 1.0 ng/mL for CBD and OH-THC. The method proved to be linear and accurate for all analites (range 1-100 ng/mL). Precision was examined in term of inter- and intra-assay variability. Measurement uncertainties were also evaluated, and decision rules were set with confidence for forensic purposes. Method performances were monitored over a long-term period and tested on thousands DUID samples, including about 750 positive samples.

Conclusions: The fully validated method was simple, fast, highly specific, and sensitive. It may become suitable for clinical and forensic toxicology applications, taking advantage of the small matrix volume required, the simple and cost-effective sample preparation procedure, and the fast analytical run time.

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