

## **Screening of biofluids for detection of drugs of toxicological interest by online extraction using turbulent flow chromatography and high resolution/accurate mass hybrid quadrupole Orbitrap mass spectrometry**

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Nowadays toxicology laboratories must be able to identify a very large number of drugs of toxicological interest and/or their metabolites in biological fluids. Hence, they need to evolve from classic, yet time-consuming, off-line liquid-liquid extraction (LLE) or solid-phase extraction (SPE) sample workup procedures to automated ones. Moreover, advanced mass spectrometric techniques, such as high resolution accurate mass hybrid quadrupole Orbitrap mass spectrometry (HRAM-Orbitrap-MS), may be employed, allowing for fast and sensitive untargeted or targeted screening procedures.

A workflow for the screening of blood, urine, and hair samples for forensic toxicology purposes using an online extraction system based on turbulent flow chromatography coupled to a new generation HRAM-Orbitrap-MS system is described.

Samples, after the addition of deuterated internal standards and a simple deproteinization step (blood and urine), or external decontamination, pulverization and solvent extraction (hair), are online processed by a Thermo Fisher Scientific (TFS) Transcend II turbulent flow chromatography (TurboFlow) system. Analytes are separated on a TFS Ultimate 3000 ultra-high-pressure liquid chromatography system equipped with a TFS Accucore Phenyl-Hexyl analytical column, and detected by a TFS Q-Exactive Focus HRAM-Orbitrap-MS system, equipped with a heated electrospray ionization (HESI)-II source. Full scan MS acquisition and subsequent fragmentation scans using the data-dependent acquisition (DDA) or variable data-independent acquisition (vDIA) mode are used. Identification of analytes is based on retention times, accurate mass measurements of their  $MH^+$  ions, evaluation of  $MH^+$  isotopic patterns, detection of accurate masses of  $MH^+$  collision-induced product ions.

Injection of high amounts of samples, due to TurboFlow technology, together with the high analytical specificity and sensitivity attainable with the Q-Exactive Focus HRAM-Orbitrap-MS system, permit to process low amounts of biofluids (blood and urine, 50  $\mu$ L; hair 5-10 mg). Total run time per sample is 20-30 (blood, urine) and 40 (hair, barring solvent extraction) min. The developed workflow can be applied to detect traditional illicit drugs, novel psychoactive substances, and pharmaceuticals of toxicological interest and/or their metabolites for numerous forensic applications, e.g. in the fields of driving under the influence of drugs and drug-facilitated crimes.