K52 The Screening of Biofluids for the Detection of Traditional Illicit Drugs, New Psychoactive Substances (NPS), and Pharmaceuticals by Automated Online Extraction Using Turbulent Flow Chromatography and High Resolution Accurate Mass-Hybrid Quadrupole-Orbitrap-Mass Spectrometry (HRAM/Q-OT/MS)

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After attending this presentation, attendees will understand the advantages of performing toxicological screening of biofluids using automated online extraction coupled with HRAM/Q-OT/MS for the detection of traditional illicit drugs, NPS, and pharmaceuticals of toxicological interest.

This presentation will impact the forensic science community by providing and demonstrating the applicability of a workflow for a targeted screening of blood, urine, and hair samples for forensic toxicology purposes.

Clinical and forensic toxicology laboratories worldwide are analytically challenged by the unceasing spread of traditional illicit drugs, the increasing number of NPS, and the misuse of prescription medications. Therefore, these laboratories are increasingly required to provide fast, comprehensive, and highly sensitive screening protocols for identifying a large number of drugs of toxicological interest and/or their metabolites in biological fluids. Hence, these laboratories need to evolve from classic, yet time-consuming, off-line Liquid-Liquid Extraction (LLE) or Solid-Phase Extraction (SPE) sample workup procedures to automated procedures. Moreover, in addition to traditional chromatographic and mass spectrometric techniques (Gas Chromatography/Mass Spectrometry (GC/MS), Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS), and Liquid Chromatography/Mass Spectrometry (LC/MS/MS)), advanced MS techniques, such as LC coupled with HRAM-Q-OT/MS, may be employed, allowing for simple, fast, and sensitive untargeted or targeted screening procedures.

The purpose of this presentation consists in the description of the workflow developed at an Italian laboratory for a targeted screening of blood, urine, and hair samples for forensic toxicology purposes using an automated on-line extraction system based on turbulent flow chromatography coupled to a new-generation HRAM-Q-OT/MS system.

After the addition of deuterated internal standards and a simple deproteinization step (blood and urine), or external decontamination, pulverization, and solvent extraction (hair), samples were processed on-line by a Transcend™ II turbulent flow chromatography (TurboFlow) system. Analytes were separated on an UltiMate® 3000 Ultra-High-Pressure Liquid Chromatography (UHPLC) system equipped with an Accucore™ Phenyl-Hexyl analytical column, and detected by an Q Exactive™ Focus HRAM-Q-OT/MS system, equipped with a Heated Electrospray Ionization (HESI) -II source. MS acquisition was performed using positive/negative switching in full scan mode at a resolution of 35,000 and subsequent Data-Dependent Acquisition (DDA) mode, performing High-

energy Collisional Dissociation (HCD) experiments at a resolution of 17,500 according to dynamic exclusion and inclusion lists on the masses of interest. Identification of analytes was based on accurate mass measurements of their MH^+ ESI-generated ions in full scan conditions, evaluation of MH^+ isotopic patterns, detection of accurate masses of MH^+ collision-induced product ions, and comparison with full HR-MS/MS library spectra.

The developed workflow proved to be fast, reliable, and highly sensitive. Total run time per sample, including minimal sample pretreatment, TurboFlow processing, and HRAM-Q-OT/MS analysis, ranged from 20-30min (urine, blood) to 40min (hair, barring solvent extraction). This workflow was applied to detect traditional illicit drugs, NPS (mainly phenethylamines, tryptamines, piperazines, cathinones, synthetic cannabinoids), and pharmaceuticals of toxicological interest and/or their metabolites in blood, urine, and hair samples for applications in the fields of Driving Under the Influence (DUI) of drugs, Workplace Drug Testing (WDT), drug-facilitated crimes, and Postmortem (PM) toxicology. Representative analytical findings from selected forensic toxicology cases will be presented and discussed (e.g., DUI cases in which THC and metabolites (Case 1) or cocaine, oxycodone, venlafaxine, quetiapine, diazepam, and/or their metabolites (Case 2) have been detected/quantified in urine and blood) demonstrating the usefulness of the described analytical approach in replacing immunoassay screening. Discussion will include a fatality case in which heroin, cocaine, levamisole (cocaine adulterant), fluoxetine, promazine, diazepam, and/or their metabolites were detected/quantified in PM urine, blood, and vitreous humor and a driving licence regranting case in which trazodone and its metabolite m-chlorophenylpiperazine were detected in hair collected from a driver wrongly charged with DUI of amphetamines merely on the basis of a positive immunoassay urine screening.

Forensic Toxicology, Biofluids Screening, High Resolution MS