K28 An Easy, Fast, and Reliable Workflow to Perform Real Forensic/Toxicological General Unknown Screening

Adrian M. Taylor, PhD, 71 Four Valley Dr, Concord, ON L4K 4V8, CANADA*

After attending this presentation, attendees will learn about a comparative screening workflow that allows the comparison between a sample and control in which significant differences in the sample are automatically extracted resulting in a reduction of several hundred peaks down to identifying only the significant components of the sample. Learning outcomes will include how high resolution accurate mass instrumentation can be successfully used to provide comprehensive and valuable information in identification of unknowns. Currently real General Unknown Screening suffers from complexity of biological matrices, which makes it almost impossible to identify relevant compounds in an easy and fast way. This presentation will present an easy-to-use generic workflow for General Unknown Screening. As an example, Tramadol in urine will be shown to be easily detected with only two injections in a Comparative Screening workflow using a hybrid quadrupole/time-of-flight instrument.

This presentation will impact the forensic science community by showing a fast, confident, and easy-to-use workflow to perform real non-targeted screening. The General Unknown Comparative Screening workflow provides basic sensitivity in Mass Spectrometry (MS) and Tandem Mass Spectrometry (MS/MS) modes for a clear identification of compounds, high resolution to overcome selectivity issues, and mass accuracy to capitalize from the provided resolution.

Method: ekspert™ ultraLC device was coupled to a fast-scanning high resolution MS system providing fast and sensitive MS/MS capabilities. Information-dependant acquisition with dynamic background subtraction and dynamic exclusion triggered 10 MS/MS experiments. The resulting total cycle time ensured that the compounds had more than 10 data points across extracted ion chromatograms (peak width 4 – 5 sec). Total LC runtime was 10 minutes using 6 min gradient (95% de-ionised water to 0% de-ionised water) with Phenomenex Kinetex 2.6µm C18 Column, 100 Å, 50 x 2.1mm column.

Results: The Comparative Screening workflow required two injections; a control injection was followed by the sample injection. The control was a urine sample of approximately comparable matrix to the sample without any drugs. Both data were loaded into PeakView™ software and automatically evaluated by an additional software add-on. All peaks overcoming a defined threshold were evaluated for retention time similarities in both sample and control. Significant differences in the sample due to, for example, absence or lower abundance of the same peak in control were automatically extracted and MS as well as MS/MS information was displayed (defined by second threshold). Thus, a reduction of several hundred peaks down to what is specific to the sample only were identified; Tramadol and its related major metabolites (demethylation). Sensitive MS/MS information can be used for confident identification by automatic searching MS/MS forensic library (1,250 entries). In case of missing conformity of a detected mass with any compound in any library, additional built-in software tools help to identify formulas by accurate mass, isotopic ratio, and sensitive MS/MS information. Finally, potential structures of calculated formula can be verified by fragment-predictive software tools.

Comparative General, LC/MS/MS, Accurate Mass

 ** Presenting Author*