

# THE DEVELOPMENT OF A TOP-DOWN PROTEOMIC METHOD FOR THE CONFIRMATORY IDENTIFICATION OF SALIVA AND SEMEN BY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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Modern molecular and spectroscopic techniques have emerged to supplant traditional enzyme- and antibody-based tests for the identification of body fluids. These techniques aim to bring technological advances to serological analysis in an attempt to match the strides made in DNA/STR testing. One approach, proteomics and protein mass spectrometry, has seen success in the past decade. However, multi-stage workflows (e.g. protein extraction, quantitation, enzymatic digestion, and solid phase cleanup) associated with proteomic analysis remain a major hurdle towards the adoption of the technique in caseworking laboratories. Here, a streamlined sample-to-results workflow has been developed using peptidomics or “top-down” protein-level analysis, allowing for straightforward sample preparation versus traditional enzymatic digestion or “bottom up” methodologies.

Low molecular weight proteins were extracted using Waters Oasis® HLB cartridges. Data acquisition was performed on an AB SCIEX™ TripleTOF® 5600 platform coupled to a Shimadzu Nexera XR UHPLC, and biomarker candidates were identified using ProteinPilot™ software. To date, numerous candidate biomarkers have been characterized in both human seminal fluid and saliva. This includes well known proteins such as Semenogelins I and II proteins in seminal fluid and Submaxillary Gland Protein 3B and Statherin proteins in saliva. In conclusion, the research presented will demonstrate the use of intact protein analysis for the confirmatory identification of saliva and seminal fluid in forensic-type samples.