

**PT22 The Development of an MRM Assay for Quantitation of low abundance cytochrome P450 proteins**

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Proteomics, MS<sup>E</sup>, MRM, Quantitation

Using a novel Workflow can improve the specificity of peptide MRM's when used in a discovery experiment

The expression ratio of endogenous Cytochrome P450 proteins from liver cells change between control/disease states. A nonbiased LC MS/MS strategy, Identity<sup>E</sup> using QToF mass spectrometry produces a list of proteins and associated peptides. The high energy MSe information is used to build quantitation methods for specific CYP 450 peptides which are monitored using triple quadrupole mass spectrometry by multiple reaction monitoring (MRM). MRM analysis is robust and provides specificity, selectivity and the sensitivity required for low abundance peptides. The sensitivity MRM analysis improved by coupling nanoscale UPLC to the mass spectrometer, delivering separated components at high peak concentrations to the ionization source at reproducible retention times. MRM transitions and acquisition parameters, including, accurate retention time windows, CV and CE were determined from the Identity<sup>E</sup> experiments.

CYP450 enzymes play a major role in oxidative metabolism and are inhibited or induced by drugs, foods and diseases. The sequence homology between the CYP450 isoforms represents a challenge for MRM method development.