A HIGHLY MULTIPLEXED MRM-BASED SCREENING ASSAY FOR PRECLINICAL NEPHROTOXICITY STUDIES

OBJECTIVE – Develop an Ab-free, highly multiplexed, mass spectrometry MRM-based assay to support evaluation of drug-induced nephrotoxicity in preclinical rat studies, with the following characteristics:

- Includes FDA and EMEA-qualified biomarkers
- Includes additional or complimentary markers
- Easily and quickly customized with additional/suspected markers
- Value-added economical assay useful in characterizing the nature of a nephrotoxic insult

METHOD – Known and suspected biomarker candidates for nephrotoxic injury were identified via literature database search. An LC-MRM-based assay was developed for use in screening of candidate biomarker expression levels in urine or plasma. A sample method for urine was optimized and described. The LC-MRM assay was then tested on rat urine and used to screen biomarker expression levels in urine from a rat model of nephrotoxic-induced kidney injury.

RESULTS –

DISCUSSION AND FUTURE STUDIES – Caprion is ready to offer services for a panel of 29 nephrotoxic safety biomarkers, for differential intensity-based screening relative to control. Services presently under development include absolute quantitation using heavy atom labeled peptides, and application of our safety biomarker panel towards clinical drug development studies.

BACKGROUND

TECHNOLOGY PLATFORM – Mass Spectrometry has been a workhorse in the small molecular world for decades, for routine quantitation and screening; determining the composition of a molecule and providing structural insights using mass spectral fragmentations.

Triple quadrupole mass spectrometry, interfaced with HPLC offers the following characteristics for the high-throughput analysis of complex biological matrices:

- Specificity
- Sensitivity
- Multiplexing Capability

From small amounts of complex biological matrix, multiple precision readings can be quickly obtained from a single run, on hundreds of targets.

- Molecular Mass
- Sequence/Structure
- Identity/Quantity

Triple quadrupole mass spectrometry provides a reliable and established alternative platform to ELISA and other indirect, antibody based assays. The resolving and filtering power of quadrupole mass analyzers allows for the direct selection, isolation and measurement of target molecules. Any protein or peptide can be targeted, so long as the target genomic or amino acid sequence is known. Assay development time for new targets is on the scale of weeks and does not rely on antibodies.

METHOD

BIOMARKER TARGET SELECTION

- Generate comprehensive list of qualified and potential biomarkers
- Select up to 5 peptides to represent each protein
- Obtain synthetic peptides for testing and optimization

Multiple MRM assay optimization

- Select synthetic target peptide endpoint
- Generate MRM assay matrix for a triple quadrupole mass spectrometer
- Validate synthetic peptide standard curve
- Establish optimal HPLC/MS parameters coupled method for a triple quadrupole mass spectrometer
- Establish optimal UHPLC/MS parameters coupled method for a triple quadrupole mass spectrometer

ASSAY FORMAT

Sample vol: 50-100UL
Format: TURK (n=30)
96 well plate (n=30)

ASSAY VOLUME

200UL rat urine

ASSAY SAMPLE PREPARATION

- Precipitate solution
- Incubate
- Washes
- Trypsin digestion
- Resulting

REPRODUCIBILITY TESTING: NORMAL POOLED RAT URINE

- Pooled, normal rat urine was used to test assay performance and processed using two formats: plate-based (n=72) and tube-based (n=20)
- Each test sample was spiked with a mixture of internal standard synthetic peptides prior to LC-MRM analysis, to enable performance monitoring at the instrument level

PROOF-OF-CONCEPT STUDY DESIGN: RAT MODEL OF XENOBIOTIC-INDUCED KIDNEY INJURY STUDY SAMPLES

Rats were treated with a chemotherapeutic agent known to cause nephrotoxicity.

- HIGH DOSE
- MEDIUM DOSE
- LOW DOSE
- VEHICLE GROUP
- ABEASE

QC SAMPLES AND CONTROLS

- Pooled, normal rat urine was used as QC control
- 10 QC controls were used for all 48 study samples
- All study samples and QC controls were spiked with a mixture of internal standard synthetic peptides prior to LC-MRM analysis, to enable performance monitoring at the instrument level

RESULTS

REPRODUCIBILITY TESTING OF THE LC-MRM BIOMARKER PANEL IN RAT URINE

A aliquots of pooled rat urine (50-100UL each) was processed using either a plate-based (n=72) or tube-based (n=20) protein precipitation method, then digested using trypsin, and spiked with internal standard synthetic peptides. Samples were then analyzed using the multiplexed biomarker panel, with more than 200 peptide fragmentation transitions monitored in each run, thus allowing for detection of more than 30 proteins from a single 50UL urine sample. The detected proteins included qualified biomarkers, biomarkers undergoing qualification, and exploratory biomarkers. Assay performance metrics are provided for process-related and instrument-related variation below, plate-based and tube-based sample preparation formats.

PROCESS-RELATED REPRODUCIBILITY

In a separate round of process-related variability, the biomarker panel was run 10 times on both plate and tube formats for each peptide. Three different lots of reagents were used and each lot was used two times, for a total of 30 runs. The RSD was determined in triplicate. The data was processed using the caprion.com software and compared to values obtained with the BioPharma Assay Kit v2.

LC-MRM-RELATED REPRODUCIBILITY

To assess instrument-related variability, the biomarker panel was run 10 times on both plate and tube formats for each peptide. Three different lots of reagents were used and each lot was used two times, for a total of 30 runs. The RSD was determined in triplicate. The data was processed using the caprion.com software and compared to values obtained with the BioPharma Assay Kit v2.

QUALIFIED BIOMARKERS

FDP, EMDA, PDMA, PSS

PROOF-OF-CONCEPT STUDY: RAT MODEL OF XENOBIOTIC-INDUCED KIDNEY INJURY

Peptides detected in normal rat urine by targeted LC-MRM screening

Peptide

Acetyl renin

Acetyl angiotensin II

Acetyl angiotensin I

Acetyl dehydro-renin

Acetyl angiotensin 1-7

Acetyl angiotensin 12-7

Acetyl kininogen

Acetyl kininogen fragment

Aspartate aminotransferase

Biochemical assays

Caprion is ready to offer services for a panel of 29 nephrotoxic safety biomarkers, for differential intensity-based screening relative to control. Services presently under development include absolute quantitation using heavy atom labeled peptides, and application of our safety biomarker panel towards clinical drug development studies.

For more information on the biomarker panel or other services, please contact info@caprion.com or go to www.caprion.com