

## BIOANALYTICAL SUPPORT

Sensitive analytical methods are a prerequisite for the accurate measurement of low concentrations of test compound in assay samples. Because of its sensitivity and specificity, LC-MS/MS is the technique of choice for quantitative analysis in support of ADME-Tox studies. Cerep uses state-of-the-art LC-MS/MS instrumentation to ensure that robust and reliable analytical methods are used for the quantitative and qualitative analysis of analytes in a wide range of sample matrices. Cerep's bioanalytical technologies include:

- ▶ Triple-Quadrupole LC-MS/MS (Thermo Electron)
- ▶ Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI)
- ▶ HTS PAL autosamplers for high-throughput sample analysis
- ▶ High Pressure and Ultra-High Pressure Liquid Chromatography (HPLC and UHPLC)
- ▶ HPLC-UV with photodiode array detection (Dionex)

### ANALYTICAL METHOD DEVELOPMENT

For those compounds to be tested in assays requiring MS support, analytical methods are developed in two separate protocols depending on the type of assay and/or the characteristics of the test compound. The HPLC-MS Screen assay provides the basic information necessary for selected reaction monitoring (SRM) of the test compound in general conditions compatible with high-throughput analysis. Compounds requiring the highest possible sensitivity or which have unique physical or chromatographic properties will undergo method development in the HPLC-MS optimization assay.

#### ▶ HPLC-MS SCREEN

In this assay the basic parameters needed for the tandem MS detection of a test compound are identified. A solution of the test compound is injected and analyzed by HPLC-MS (full-scan) in both positive and negative ionization mode for the identification of the pseudo-molecular ion of the test compound (i.e., protonated, deprotonated, or other species that are directly correlated to the molecular weight of the test compound). The yield of the pseudo-molecular ion in the positive or negative ionization mode in the MS spectra determines the choice of the precursor ion in the MS/MS experiment. The selected precursor ion is fragmented via collision-induced dissociation (CID) under a sequence of collision voltages, and the most abundant product ion is identified. The precursor ion – product ion pair that is identified in the MS/MS experiment becomes characteristic of the test compound and is a prerequisite for the quantitation of the test compound via selected reaction monitoring in any assay matrix. MS parameters and chromatographic conditions are not optimized in this protocol; although this approach is suitable for most compounds it is sometimes necessary to develop a customized method for better sensitivity (HPLC-MS Optimization).

#### ▶ HPLC-MS OPTIMIZATION

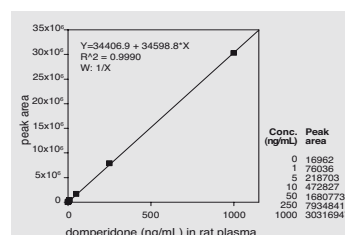
In this assay the settings for multiple MS parameters and the chromatographic conditions are optimized to give the best possible detection of the test compound. A solution of each test compound is prepared as specified and infused into the MS source via syringe pump at a constant rate. Following identification of the specific selected reaction monitoring (SRM) transition to be used for each test compound, the detection parameters are optimized using the automated protocol in the TSQ Quantum Compound Optimization workspace. Finally, the chromatographic conditions to be used for LC-MS analysis are identified by injection and separation of the analyte on a suitable LC column and adjustment of the gradient conditions as necessary.

### BIOANALYTICAL SUPPORT OF PHARMACOKINETIC STUDIES

*In vivo* PK and BBB studies begin with an initial method development phase, which includes the HPLC-MS optimization and the linearity assays. The HPLC-MS optimization assay provides the parameters for mass spectrometry (MS) detection and customizes MS settings and LC conditions for maximum sensitivity. Once the analytical method is established, samples are processed for HPLC-MS/MS (or HPLC-MS) analysis in the linearity assay. Acetonitrile is typically used to precipitate the proteins in plasma samples although liquid-liquid extraction or solid-phase extraction can be used if necessary. The linearity assay determines the linear response of the compound over a range encompassing several orders of magnitude concentration and also identifies the lower limit of quantification. The results from this assay indicate whether further method development will be necessary to obtain a cleaner extraction of the compound from the matrix with a different sample preparation method.

#### ▶ LINEARITY

This assay is performed in order to observe the relationship between instrument response and known concentrations of analyte over several orders of magnitude. A calibration curve is constructed by analysis of compound spiked into the specific matrix. From this curve the linear range of the analytical method can be determined and the lower limit of quantitation specified. Results from this assay indicate whether the sample preparation and analytical methods have the requisite sensitivity and linear range to obtain reliable data from analysis of unknown samples (Fig. 1).



**Fig. 1** Calibration curve for domperidone in rat plasma

► RECOVERY

Recovery experiments, in support of *in vivo* pharmacokinetics (PK) and blood-brain-barrier (BBB) studies, are designed and performed to estimate proportional systematic errors. These errors are often caused by one or more components of the sample matrix which interfere with detection of the analyte of interest. In this assay, two samples are prepared in a parallel step-wise protocol, with the only difference being the point in the process where the standard analyte is introduced. The calculated recovery is directly correlated to the efficiency of the extraction procedure. Results of the recovery assay are used to assess whether the sample preparation method is suitable for a given analyte, or whether further method development is required. Typically the Recovery assay is included in the analytical method development process only if a sample preparation method other than acetonitrile precipitation is needed (Fig. 2).

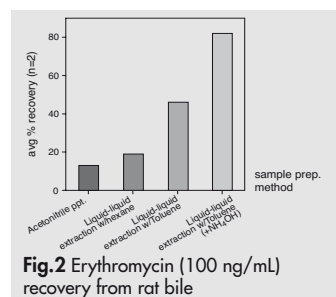


Fig.2 Erythromycin (100 ng/mL) recovery from rat bile

► QUANTITATIVE BIOANALYSIS

In this assay the concentration of analyte in unknown samples is determined. Plasma, blood or tissue samples collected from dosed animals are processed and analyzed simultaneously with spiked standards. A calibration curve is constructed from the standards and used to calculate the concentration of analyte in the unknown plasma or tissue samples. Available matrices: Plasma, blood, bile, urine, brain or liver from any species.

■ DRUG EXPOSURE MEASUREMENT / DOSE SOLUTION ANALYSIS

Although the assays described here are commonly performed in support of Cerep's in-house pharmacokinetic studies, this general approach can be also applied as a stand-alone protocol with samples obtained in client or third-party studies (Drug exposure measurement). Similarly quantitative bioanalysis can be included as part of ADME-Tox studies if accurate determination of the concentration of a test compound or metabolites is needed for *in vitro* assay samples. Specific examples of this include the determination of the concentration of compound in test solutions used for the hERG patch-clamp assay or in formulations used to dose animals (Dose solution analysis).

■ DELIVERABLES

- Concentration of compound expressed as ng/mL in plasma, blood, bile, and urine or ng/g in tissue
- Sample preparation and the HPLC-MS/MS method for each compound are reported.



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■ QUESTIONS OR CONCERNS?

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