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# In-Vivo Mouse and Rat PK Bioanalysis

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**Protocol status:** Working
**We use this protocol and it's working**

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## Disclaimer



## Abstract

In vivo PK bioanalysis involves studying drug behaviour within a living organism to understand how it is absorbed, distributed, metabolized, and excreted (ADME). This is crucial for drug discovery and development, providing insights into a drug's efficacy and safety before clinical trials. Bioanalysis plays a key role in accurately measuring drug concentrations in biological samples, like plasma, to assess ADME and other pharmacokinetic parameters.

## Safety warnings

Always wear appropriate PPE for this protocol
Refer to Material Safety Data Sheets for additional safety and handling information.

## Summary

1 In vivo PK bioanalysis involves studying drug behaviour within a living organism to understand how it is absorbed, distributed, metabolized, and excreted (ADME). This is crucial for drug discovery and development, providing insights into a drug's efficacy and safety before clinical trials. Bioanalysis plays a key role in accurately measuring drug concentrations in biological samples, like plasma, to assess ADME and other pharmacokinetic parameters

| | Options Run |
|---|---|
| | in-vivo mouse single dose PK IV |
| | in-vivo mouse single dose PK PO |
| | in-vivo rat single dose PK IV |
| | in-vivo rat single dose PK PO |

## Sample Preparation

2 Bioanalysis begins with the preparation of a primary stock solution at 1,000,000 ng/mL using DMSO or a suitable alternative, which is then serially diluted to generate standard and quality control (QC) working solutions from 2000ng/ml to 0.5ng/ml.

These solutions are stored at 4°C for up to one week. Calibration standards and QCs are prepared in 96-well plates, ensuring that the volume of organic solvent does not exceed 10% of the plasma to prevent protein precipitation.

3 For sample preparation, thawed biological samples are added to the plates, mixed thoroughly, and diluted if necessary. An internal standard (IS) in acetonitrile is then added before centrifugation.

## Assay

4 Tissue samples are homogenized in phosphate buffer, with dilution ratios tailored to the tissue type, and analysed using either a tissue-specific standard curve or surrogate matrix when required.

5 During LC-MS/MS analysis, 100 µL of supernatant is transferred to a new plate, diluted with 0.1% formic acid, and analysed following the generated run list.

## Data Analysis

6 Data processing involves assessing calibration curve fit, where at least 75% of calibration points must fall within ±25% of their nominal values. QC sample acceptance requires two-thirds to meet ±25% criteria, including half at each concentration level.

7 Carryover should not exceed 1% of the top standard, and any interference in blanks must remain below 20% of the lowest standard. Internal standard response variation over 30% must be reviewed by senior staff, though early run variation is typically acceptable. For unknown samples, if neat and diluted values differ by more than 20%, the diluted value is reported to account for potential matrix effects.

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- AUC (Area Under the Curve): Represents the total drug exposure over a specific time period.
- Cmax (Maximum Concentration): The highest drug concentration in the blood stream.
- t½ (Half-life): The time it takes for the drug concentration to halve.
- Vss (Volume of Distribution): The apparent volume of the body in which the drug is distributed.
- Clearance: The rate at which the drug is eliminated from the body.

## Acknowledgements

Grateful to Concept Life Sciences for supplying the original protocol steps