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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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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 μ L 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 $\pm 25\%$ of their nominal values. QC sample acceptance requires two-thirds to meet $\pm 25\%$ 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.
- 8
 - AUC (Area Under the Curve): Represents the total drug exposure over a specific time period.
 - C_{max} (Maximum Concentration): The highest drug concentration in the blood stream.
 - t_{1/2} (Half-life): The time it takes for the drug concentration to halve.
 - V_{ss} (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.

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