In-vitro Pharmacokinetics & Drug Metabolism Profiling Assays

1. Solubility of Test Item at pH 7.4

UV Spectrophotometric analysis of serially diluted solutions (six levels) from stock using ACN and buffer, to evaluate the solubility of NCE's in buffer

2a. Metabolic Stability (two time point)

1 Test Compound, 4 Species (rat, dog, monkey and human), 1 protein concentration, with and without cofactor, duplicate measurement, along with data for positive control substrate, % metabolism at 30 min.

2b. Metabolic Stability (six time point)

1 Test Compound, 4 Species (rat, dog, monkey and human), 1 substrate Concentration, 1 protein concentration, with and without cofactor, duplicate measurement, along with data for positive control substrate, % metabolism at 30 min, half life and intrinsic clearance.

3. Metabolite Identification

1 Test Compound, Microsomes from two Species (rat or mouse, human), incubated at 30 min and 2 hours, scan with LC-MS/MS for possible metabolites.

4. CYP Inhibition

1 compound, 5 assays (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4), (8-12 test item concentrations), singlet measurement, along with $_{IC50}$ of positive control inhibitor.

5a. Protein Binding

Plasma of three species (human, dog, rat) equilibrium dialysis-5h, 1 test concentration, 3 replicate cells, estimation of concentration in plasma and buffer in single curve

5b. Protein Binding

Plasma or microsomes or rat brain homogenate in equilibrium dialysis-5h, 1 test concentration, 3 replicate cells, estimation of concentration in plasma and buffer in single curve

6. Brain in Beaker

Concentration gradient to draw small molecules out of brain homogenate. The brain

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homogenate is in dialysis membrane compartment and is spiked with up to 4 test compounds and a standard, quality control compound, quantitation using LC-MS/MS system.

7. Time dependent inhibition

1 compound, 5 assays (1A2, 2A6, 2B6, 2C9, 2C19, 2D6, and 3A4), (1 test item concentration), triplicate measurement, with percent inhibition IC_{50} of positive control inhibitor.

8. IC_{50} shift assay

1 compound, 5 assays (1A2, 2A6, 2B6, 2C9, 2C19, 2D6, and 3A4), (8 test item concentration), singlet measurement with three curves (0 min pre-incubation, 30 min pre-incubation without NADPH and 30 min pre-incubation with NADPH, with IC_{50} fold shift

9. Distribution coefficient

UV Spectrophotometric analysis of test compound in octanol phase and buffer phase to evaluate the distribution coefficient at pH 7.4.

10. MAO A & B Inhibition

1 compound, 2 assays (MAO – A & B), (8-12 test item concentrations), singlet measurement, along with IC_{50} of positive control inhibitor.

11. Reaction phenotyping

1 Test Compound, human liver microsomes and recombinant CYP, 1 protein concentration, with and without inhibitor, duplicate measurement, with percent contribution of each enzyme.