

TOXICITY AND GENOTOXICITY STUDIES

1.0 Acute Toxicity Studies

1.1 Acute Oral Toxicity and Toxicokinetic Study in Mice (Swiss Albino Mice)

In order to determine LD₅₀ (cut-off) of test item, mice will be dosed with single dose of test item at different dose levels. Different parameters like clinical signs, mortality and body weight will be recorded during the study period. Detailed macroscopic examination will be performed for found dead or terminally sacrificed animals. Satellite groups for toxicokinetic will be maintained to determine test item exposure. Study will be conducted as per OECD guideline 423. (LC-MS/MS method development and one day validation charges as defined in 4.0)

1.2 Acute Oral Toxicity and Toxicokinetic Study in Rats (Wistar / Sprague-Dawley)

In order to determine LD₅₀ (cut-off) of test item, rats will be dosed with single dose of test item at different dose levels. Different parameters like clinical signs, mortality and body weight will be recorded during the study period. Detailed macroscopic examination will be performed for found dead or terminally sacrificed animals. Satellite groups for toxicokinetic will be maintained to determine test item exposure. Study will be conducted as per OECD guideline 423. (LC-MS/MS method development and one day validation charges as defined in 4.0)

2.0 Repeated Dose Toxicity Studies

2.1 4-Days Repeated Dose Oral Toxicity and Toxicokinetics Study in Rats (Wistar / Sprague Dawley)

To evaluate repeated dose toxicity of test item, rats will be dosed at four dose levels for 4 days. Different parameters like mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, coagulation parameters, urine

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analysis, organ weights, detailed macroscopic examination and histopathology of major organs will be studied. Satellite groups will also be maintained to determine toxicokinetic parameters on day 1 and day 4. MTD and NOAEL will be determined based on the findings. (LC-MS/MS method development and one day validation charges as defined in 4.0)

2.2 7-Days Repeated Dose Oral Toxicity and Toxicokinetics Study in Rats (Wistar/Sprague Dawley)

In order to determine toxicity of test item, rats will be dosed at four dose levels for 7 days. Body weight and food consumption will be measured during study period. Hematology, clinical chemistry, coagulation and urine parameters will be evaluated. Detailed gross necropsy and histopathology of major organs will also be performed. Satellite groups will be maintained for toxicokinetic analysis. Based on all parameters, MTD and NOAEL will be provided. (LC-MS/MS method development and one day validation charges as defined in 4.0)

2.3 14-Days Repeated Dose Oral Toxicity and Toxicokinetics Study in Rats (Wistar/Sprague Dawley)

To evaluate repeated dose toxicity of test item, rats will be dosed at four dose levels for 14 days. Different parameters like mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, coagulation parameters, urine analysis, organ weights, detailed macroscopic examination and histopathology of major organs will be studied. Satellite groups will also be maintained to determine toxicokinetic parameters on day 1 and day 14. MTD and NOAEL will be determined based on the findings. (LC-MS/MS method development and one day validation charges as defined in 4.0)

2.4 28-Days Repeated Dose Oral Toxicity and Toxicokinetics Study in Rats with Recovery Period (Wistar/Sprague Dawley)

In order to determine sub-acute toxicity of test item, rats will be dosed at four dose levels for 28 days. Different parameters like mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, coagulation parameters, urine analysis, organ weights, detailed macroscopic examination and histopathology of all organs (control and high dose groups) will be studied.

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Reversal of test item related effects would be evaluated. Satellite groups will also be maintained to determine toxicokinetic parameters on day 1 and day 28. (LC-MS/MS method development and one day validation charges as defined in 4.0)

3.0 Genotoxicity Studies

3.1 *In Vivo* Mammalian Erythrocyte Micronucleus Test in Swiss Albino Mice

To evaluate genotoxic potential of test item, mice will be dosed at six dose levels for 2 days including positive and negative controls. Bone marrow of femur bones will be harvested and slides will be prepared and scored for presence of micronucleus in polychromatic erythrocytes. Test item concentration at different time points will also be determined from satellite groups. (LC-MS/MS method development and one day validation charges as defined in 4.0)

3.2 *In Vivo* Chromosome Aberration Test in Swiss Albino Mice

To evaluate genotoxic potential of test item, mice will be dosed at six dose levels for 2 days including positive and negative controls. Bone marrow of femur bones will be harvested and slides will be prepared and evaluated for presence any numeric as well as structural damage in chromosome. Test item concentration at different time points will also be determined from satellite groups. (LC-MS/MS method development and one day validation charges as defined in 4.0)

3.3 *In Vivo* Chromosome Aberration Test in Rats

In order to determine genotoxic potential of test item, rats will be dosed at six dose levels for 2 days including positive and negative controls. Bone marrow of femur bones will be harvested and slides will be prepared and evaluated for presence any numeric as well as structural damage in chromosome. Test item concentration at different time points will also be determined from satellite groups. (LC-MS/MS method development and one day validation charges as defined in 4.0)

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4.0 LC-MS/MS Method for Toxicokinetic Sample Analysis

LC-MS/MS Method development for quantitation of analyte of interest in plasma and or urine or tissues and partial validation including linearity, accuracy and precision (one P & A Batch), Recovery, Stock solution stability (6 hr & 24 hr), one week matrix stability.

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