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High Throughput LC/MS/MS Determination of AN3365, A Novel Boron-Containing Compound in Various Biological Matrices to Support Non-GLP Discovery and Pre-clinical Pharmacokinetic Studies

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Introduction

AN3365 is a member of a novel class of boron-based benzoxaborole molecules (Figure 1) and it inhibits leucy-IRNA synthetase via a unique mechanism of action¹. AN3365 has successfully completed phase I clinical trial and is moving to phase II clinical development. To support various non-GLP discovery and pre-clinical studies a sensitive and high throughput LC/MS/MS method was developed and it was successfully used for the determinations of AN3365 in mouse, rat, monkey, dog and rabbit plasma. Use of this method ensured the accuracies of pharmacokinetic, toxicokinetic and species scaling up studies of AN3365.

Methods

Standard samples of AN3365 and QC samples were prepared by serial dilutions of standard or QC sub-stock solution (200 µg/mL) with drug-free plasma. An internal standard working solution (ISWS) of 500 ng/mL was prepared by accurately diluting the sub-stock with methanol and it was kept at 4 °C before use.

Study samples and QC samples were thawed at room temperature, mixed thoroughly. A 96-well vacuum manifold was set up by putting a Strata® protein precipitation (PPT) plate (Phenomenex, CA) on the top of a 96-deep well collection plate. To each well of a PPT plate, 200 µL of cold ISWS was added (except the double blank wells where 200 µL of methanol were added). To each of the designated wells, 35 µL of drug free plasma, standard, QC or dosed experimental plasma sample was added. The plate was allowed to sit for at least 5 minutes for complete precipitation and then the vacuum was applied gradually till each well of the plate was relatively dry. The 96-deep well collection plate was removed from manifold and 100 µL of 0.1% formic acid aqueous solution was added to each well. The plate was then capped, vortexed briefly, and 10 µL of extract was injected onto an Atlantis® T3 column, 2.1 x 50 mm, 5 µm (Waters, MA) for LC/MS/MS analysis. A gradient program with 0.1% formic acid in HPLC water as mobile phase A and 0.1% formic acid in methanol as mobile phase B was applied. An ABI Sciex API 4000 linear ion TRAP quadrupole mass spectrometer (4000 Q TRAP), operated in positive turbo electrospray ionization (ESI) mode, was used for mass detection and analysis.

Multiple reaction monitoring (MRM) was used to monitor the product ion transitions of m/z 238.1 → 201.5 and 241.1 → 205.2 for AN3365 and IS, respectively. Dwell time for both transitions was 150 ms. The ESI ion source temperature was at 550 °C. Other optimized MS/MS parameters were: curtain gas flow: 20 psi; collisionally activated dissociation (CAD) gas setting: medium; ion spray voltage: 5500 V; ion gas 1 and 2: 50 psi; entrance potential: 10 V; collision cell exit potential: 16 V for AN3365 and 14 V for IS; declustering potential: 51 V for AN3365 and 86 V for IS; and collision energy: 17 eV for AN3365 and 19 eV for IS.

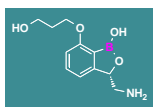


Figure 1. Chemical Structure of AN3365 (GSK2251052)

References

1. Rock, F., Mao, W., Yaremchuk, A., et al., 2007. Science, 316:1759-1761.

Results

A novel, sensitive and high throughput LC/MS/MS method was developed for the determination of AN3365 in plasma of various species. The method was linear ($r > 0.99$) over a concentration range of 1.0 - 1024 ng/mL for rodent studies and 1.0 - 2048 ng/mL for non-rodent studies, with a lower limit of quantification (LLOQ) of 1.0 ng/mL. The specificity and selectivity of the method was demonstrated by non or neglectable trace endogenous interference from the drug free plasma at retention time of AN3365 or IS, and the representative chromatograms of rat and monkey plasma were shown in Figure 2. Typical chromatograms of LLOQ and upper limit of quantitation (ULOQ) from rat and monkey plasma were presented in Figure 2. The method precision (% CV) ranged from 1.0 to 12% and accuracy (% of Nominal) were between 87.6 and 116% across mouse, rat, dog and monkey plasma (Table 1).

The non-GLP discovery and pre-clinical pharmacokinetic study and toxicokinetic samples from mouse, rat, dog and monkey were successfully analyzed using the developed method and pharmacokinetic parameters were accurately determined. The plasma concentration-time profiles of AN3365 in rat and monkey, following single IV administrations of AN3365 saline solution at 10 and 30 mg/kg, are illustrated in Fig. 3. The mean pharmacokinetic parameters estimated using a two compartmental model by WinNonlin 5.2 are summarized in Table 2. Following single IV 10 mg/kg dosing of AN3365 saline solution in rat and monkey, the maximum plasma concentration (C_{max}) at 5 min was 9.91 µg/mL and 12.3 µg/mL, respectively. The clearance of AN3365 was 1686 mL/h/kg in rat and 1993 mL/h/kg in monkey, AUC_{0-∞} was 5.99 h·µg/mL in rat and 12.8 h·µg/mL in monkey, the terminal half-life was 2.97 h in rat and 4.40 h in monkey. The volume at steady state (V_{ss}) was 4689 mL/kg in rat and 3557 mL/kg in monkey, respectively.

Table 1. The Precision and Accuracy of Mouse, Rat, Dog and Monkey Assays

Species	Nominal Concentration (ng/mL)	n	Mean Calculated Concentration (ng/mL)	Precision (% CV)	Accuracy (% of Nominal)
Mouse	6	3	5.26	12	87.6
	32	3	34.3	4.4	107
	160	3	175	6.7	110
	800	3	930	7.2	116
Rat	6	4	6.34	11	106
	32	4	32.7	11	102
	160	4	164	12	102
	800	4	797	12	99.6
Dog	12	3	11.5	2.3	95.8
	60	3	59.1	2.0	98.5
	300	3	290	2.3	96.7
	1500	3	1450	6.7	96.7
Monkey	12	5	12.9	9.7	107
	60	5	67.4	1.0	112
	300	5	325	2.4	108
	1500	5	1548	7.2	103

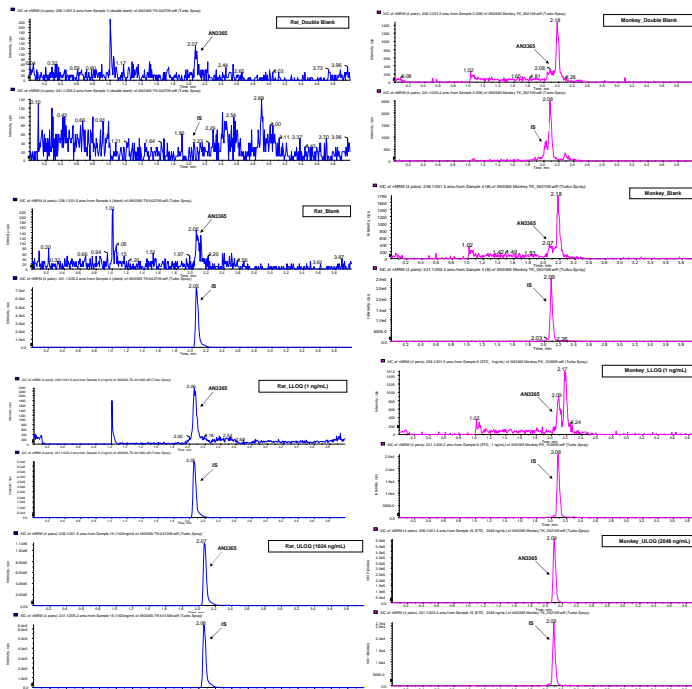


Figure 2. Representative Chromatograms of Double Blank, Blank, LLOQ and ULOQ of Rat and Monkey Assays

Table 2. Mean Pharmacokinetic Parameters of AN3365 Following Intravenous Administrations of AN3365 in Rat and Monkey

Species	Dosing Route	Dose (mg/kg)	n	Conc. @ 5 min (µg/mL)	AUC (h·µg/mL)	CL (mL/h/kg)	Terminal t _{1/2} (h)	V _{ss} (mL/kg)
Rat	IV	10	3	9.91	5.99	1686	2.97	4689
	IV	30	3	16.4	15.5	1993	2.85	4869
Monkey	IV	10	3	12.3	12.8	786	4.40	3557

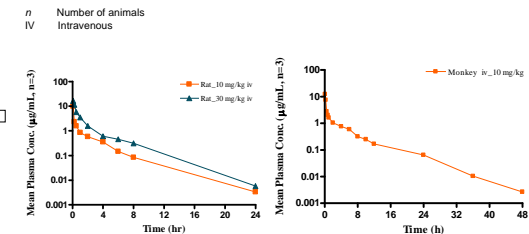


Figure 3. Plasma Concentration-time Profiles of AN3365 in Rat and Monkey Following a Single intravenous dose of 10 or 30 mg/kg

Conclusions

- A high throughput and sensitive LC/MS/MS method for the determination of AN3365 in various biological matrices was developed.
- The developed method was successfully adapted for analysis of various non-GLP PK/TK study samples.