

Establishment of a Quantitative Analytical Method for Albumin-Drug Conjugates in Rat Plasma Using Ultra-high Performance Liquid Chromatography-tandem Mass Spectrometry

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INTRODUCTION

Background

➤ Albumin is a natural transport protein for various nutrients and metabolites. The concentration of human albumin is approximately 35-50 g/L and its half-life is typically 15-21 days. Albumin is able to prolong the circulation half-life of rapidly cleared drugs and promote their accumulation within tumors. Moreover, human serum albumin is a natural protein in the human body with extremely low immunogenicity and it shows excellent biocompatibility and safety. Albumin delivery systems represent an intelligent imitation and utilization of natural physiological processes. It is crucial to evaluate the *in vitro* ADME and *in vivo* pharmacokinetic properties of albumin-drug conjugates. Few studies have been conducted on albumin-drug conjugates, possibly due to the lack of robust bioanalytical methods. In this study, we developed a quantitative analytical method for the albumin-drug conjugates. This provides technical support for studying the properties of albumin-drug conjugates.

METHOD

- The commercial MAC glucuronide phenol linked SN-38 was purchased from MCE. It's used as a test compound of albumin-drug conjugate. The maleimide will be conjugated with thiol group at cysteine34 of albumin, as illustrated in **Figure 1**.
- *In vitro* plasma stability study were performed by spiking the test compound to mouse and human plasma, set 6 time points. Also, we performed accuracy and precision run to verify reliability of method.
- *In vivo* pharmacokinetic study was performed by double doses of test compound via intravenous injection at 3 mg/kg and 10 mg/kg and two rats were euthanized at designated timepoints for plasma collection. After intravenous injection of the test compound, the maleimide group will form covalent bonds with thiol group of plasma albumin Cys34. Then the samples were extracted with cold acetone, then release SN-38 using β-glucuronidase. At last, the conjugated SN-38 were quantified by UPLC-MS/MS. The free SN-38 was extracted by protein precipitation and then quantitatively analyzed by UPLC-MS/MS.

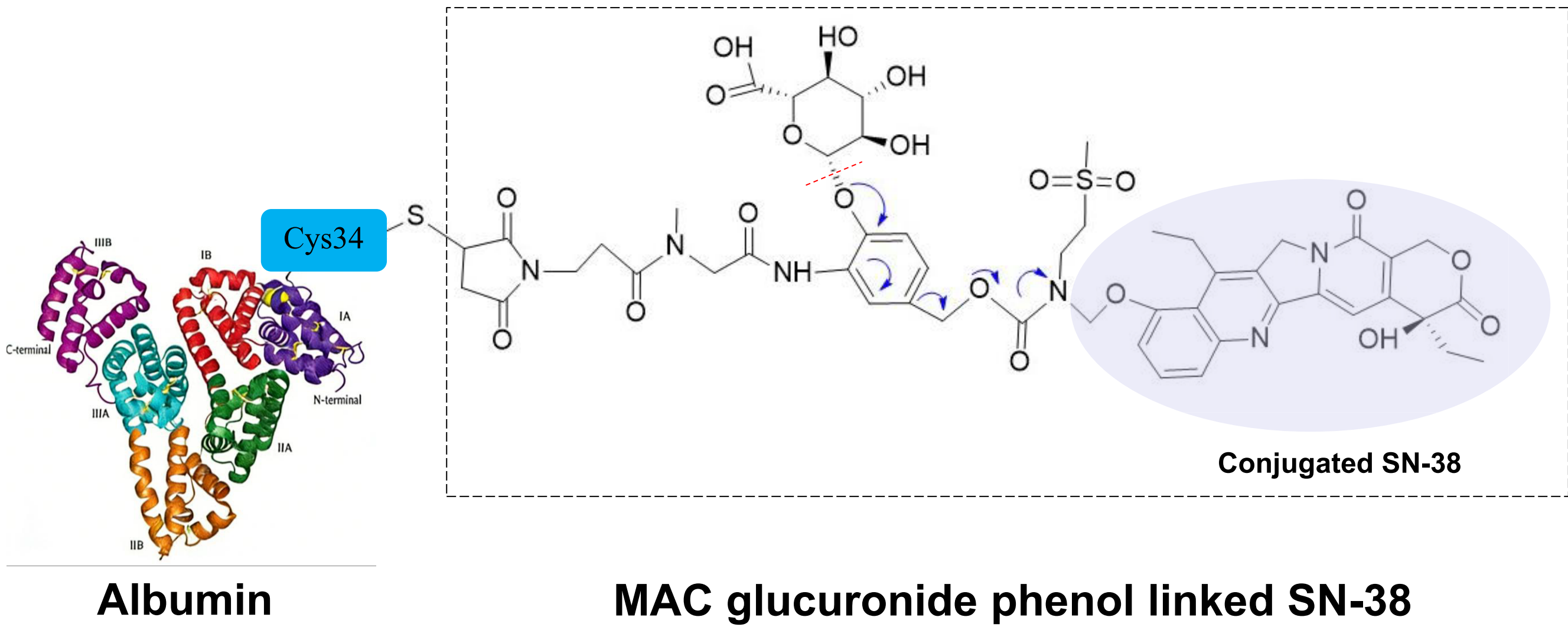


Figure 1. Michael addition reaction between thiol group and maleimide group

RESULTS

Method Validation

The assay range, accuracy and precision of the method have been comprehensively evaluated in rat plasma. The inter-day accuracy and precision of the method were assessed by analyzing QC samples at five different concentrations. As shown in **Table 1**, the accuracy and precision result shows the within-run accuracy was from 2.71% to 10.2% and precision was from -4.14% to 4.8%. The linear range of MAC glucuronide phenol linked SN-38 was from 0.005 to 10 µg/mL with excellent linearity (r > 0.99) shown in **Figure 2**. This range corresponds to SN-38 concentration of 1.8 to 3600 ng/mL. The lower limit of quantitation (LLOQ) of MAC glucuronide phenol linked SN-38 was 5 ng/mL in rat plasma, equivalent to the LLOQ of SN-38 was 1.8 ng/mL.

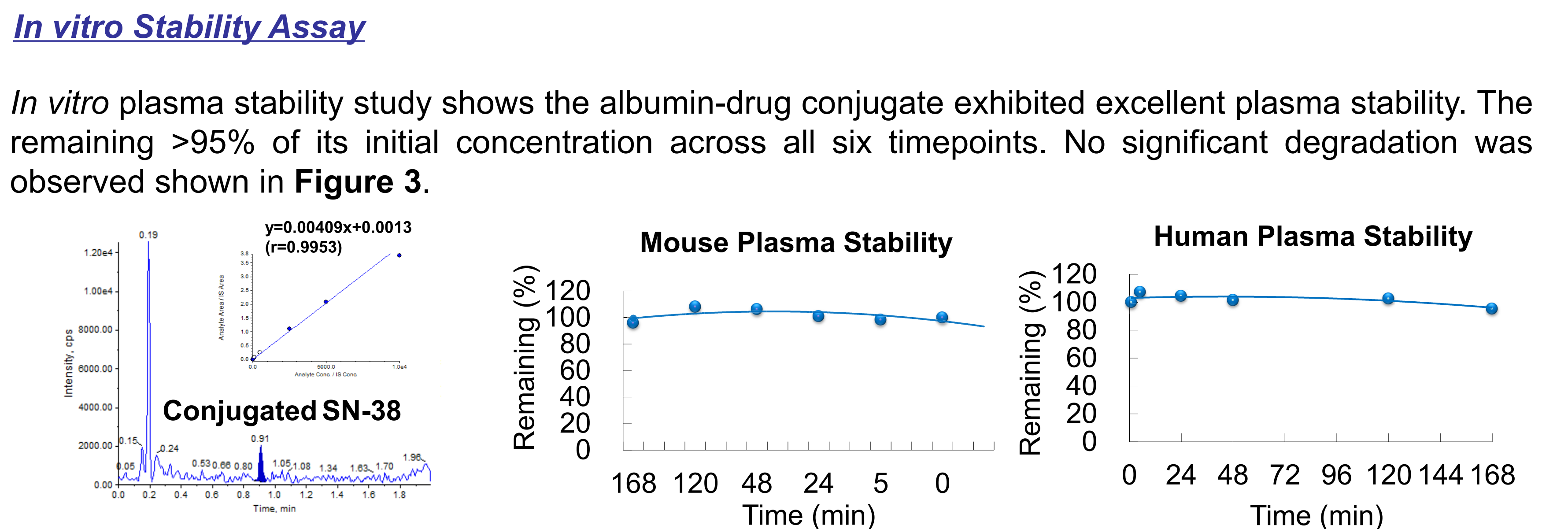


Figure 2. Chromatograms of LLOQ and calibration curve of conjugated SN-38

Figure 3. Results of mouse and human plasma stability

Table 1. Accuracy and precision of conjugated SN-38 in rat serum samples										
Assay	LLOQ (ng/mL)	%Bias	LQC (ng/mL)	%Bias	MQC (ng/mL)	%Bias	HQC (ng/mL)	%Bias	DQC (ng/mL)	%Bias
	1.80		5.39		1079		2877		28772	
Samples	1.82	1.30	5.54	2.76	1111	3.00	2949	2.50	28700	-0.250
	1.98	9.89	4.96	-7.92	1136	5.33	2892	0.500	27801	-3.37
	1.90	5.30	5.75	6.76	1090	1.00	2762	-4.00	26326	-8.50
	2.11	17.1	5.43	0.755	1061	-1.67	2884	0.250	27010	-6.12
	1.81	0.502	5.04	-6.58	1140	5.66	2676	-7.00	25571	-11.1
	1.70	-5.49	4.28	-20.6	1097	1.66	3050	6.00	30642	6.50
Within-run Mean	1.89	NA	5.17	NA	1106	NA	2869	NA	27675	NA
Within-run SD	0.143		0.529		30.0		133		1820	
Within-run %CV	7.58		10.2		2.71		4.64		6.58	
Within-run %Bias	4.78		-4.14		2.49		-0.285		-3.81	
n	6		6		6		6		6	

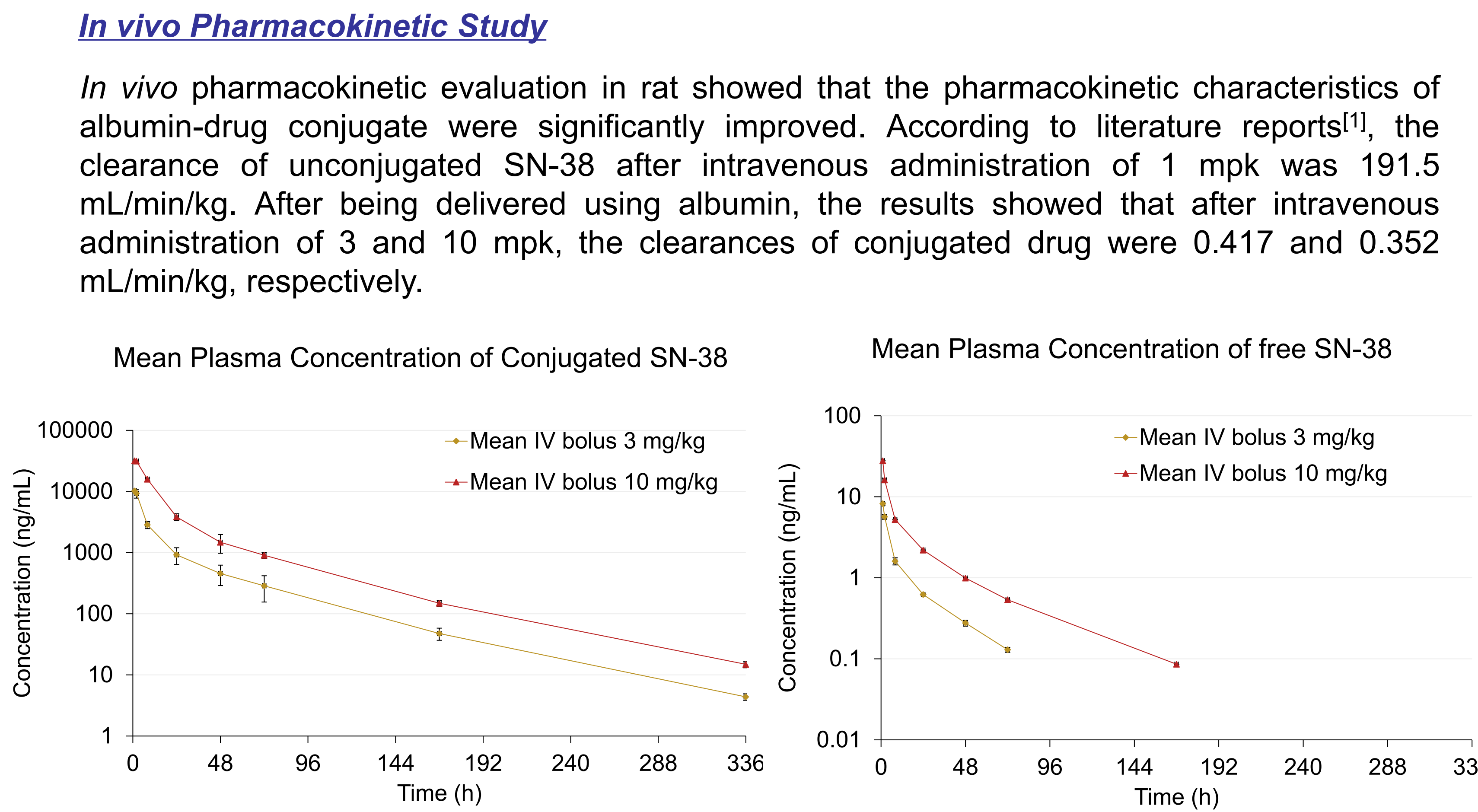


Figure 4. The PK curves of conjugated SN-38 and free SN-38

CONCLUSION

- ✓ This study established a rapid, high-throughput UPLC-MS/MS method for quantitation of albumin-drug conjugates in *in vitro* and *in vivo* biological samples. This method enables accurate measurement of conjugated and free drug concentrations in rat plasma. Moreover, the method for quantifying the conjugated drug has been fully validated, demonstrating robust sensitivity, good linearity, satisfactory intra-day precision and good accuracy. This workflow provides technical support for pharmacokinetic studies of albumin-conjugated drugs.
- ✓ In the literature^[1], albumin magnetic beads are used for enrichment which is expensive and requires a large amount of magnetic beads for each sample. In our study, cold acetone was used for extraction, which greatly improved the operability of the method. The method established in this study is a cost-effective method and well-suited for the analysis of large-scale biological samples.

REFERENCES

[1] Lim J H , Park M , Park Y ,et al. Evaluation of *In Vivo* Prepared Albumin-Drug Conjugate Using Immunoprecipitation Linked LC-MS Assay and Its Application to Mouse Pharmacokinetic Study [J].Molecules, 2023, 28(7):12

