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Practicality of the micro blood sampling (MBS) method for drug level measurement in the small animal study

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Purpose

Recently, the approach to minimize the number of animals and the pain imposed on animals are strongly required in the non-clinical study from the point of view of animal welfare. In response to this, the MBS method in the toxicokinetics study is proposed, and the discussion of this method is ongoing in ICH. In addition, the highly sensitive LC/MS/MS allows the lower limit of quantitation for drug analysis using smaller amount of blood.

In this study, the drug level measurement of a model compound, clarithromycin in single dose study in rat was carried out to confirm the validity of the method and compare the MBS and conventional sampling methods. The conditions of MBS method were also investigated.

Methods

1. Animal study

- Animal species / strain: rat / Crl:CD(SD)
- Sex / age: male / 6 weeks old (at dosing)
- Test substance: Clarithromycin
- Dosage: 200 mg/kg (single oral dosing)
- Feeding Condition: Fasted
- Examined parameters of hematology and blood biochemistry are as follows:

  Hematology: RBC, WBC, Ht, Hb, MCH, MCV, MCHC, Plt, Ret, PT, APTT, Baso, Eosi, Neut, Lymp, Mono, Eosi, Neut

  Blood biochemistry: AST, ALT, ALP, LDH, γ-GTP, Glu., T.Cho., TG, PL, TP, Alb., A/G, BUN, Crea., T.Bil., Na, K, Cl, P, Ca
- Time point (hour) of blood sampling: Pre-dosing and 1, 2, 4, 8, 24 hours post-dose
- Blood sampling method

  Micro blood sampling (n=6*): Blood samples (30 μL) were collected using winged needle and plastic capillary pre-coated with heparin sodium from jugular vein. The capillary was capped with plastic putty and centrifuged for plasma separation.

  Conventional sampling (n=9*): Blood samples (200 μL) were collected using disposable syringe pre-treated with heparin sodium from jugular vein. The blood sample was transferred to a micro tube and centrifuged for plasma separation.

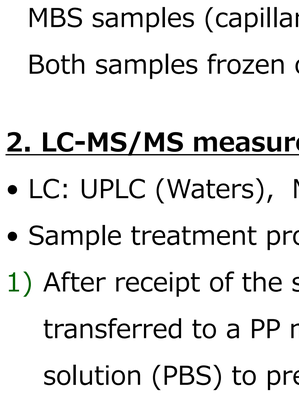
  *: For three rats, the blood sampling by both methods was carried out at the same time.
- Shipping of samples

  MBS samples (capillary) were stored in plastic test tubes to prevent the breakage in transit. Both samples frozen on dry-ice were sent to the measuring facility.

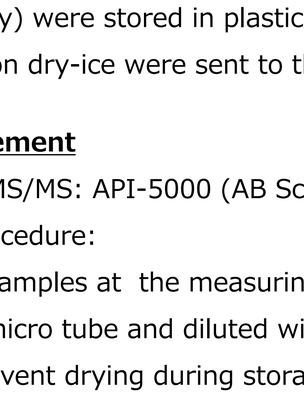
2. LC-MS/MS measurement

- LC: UPLC (Waters), MS/MS: API-5000 (AB Sciex)
- Sample treatment procedure:

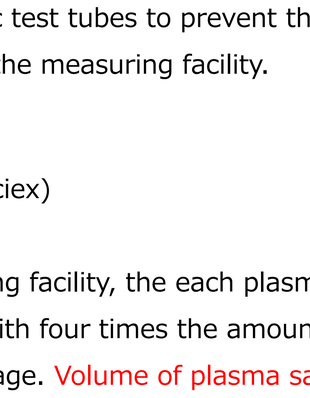
1) After receipt of the samples at the measuring facility, the each plasma sample of MBS was transferred to a PP micro tube and diluted with four times the amount of phosphate buffer solution (PBS) to prevent drying during storage. Volume of plasma sample was calculated from the weight using specific gravity. The plasma samples from the conventional method were diluted just before sample treatment (mixed 10 μL of plasma and 40 μL of PBS). The handling procedure of the capillary is shown below.
2) All diluted samples (12.5 μL, 2.5 μL as plasma) were treated by liquid-liquid extraction using *t*-butyl methyl ether. The final volume of treated sample was 150 μL.
3) Fifteen micro liter of the sample was injected into LC-MS/MS.

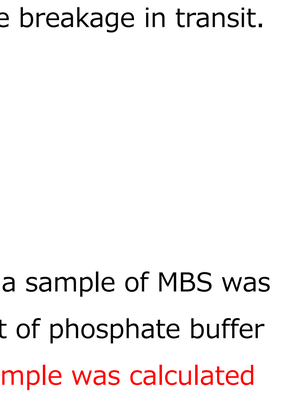


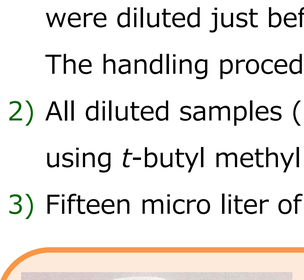
① Capillary sample in outer case (test tube)

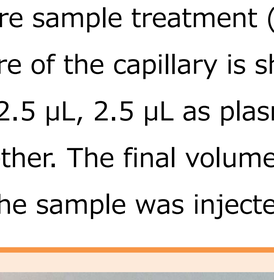


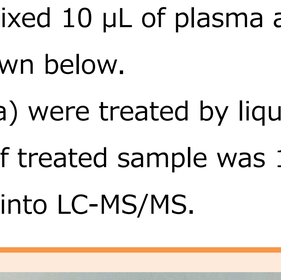
② Pulling out from the case

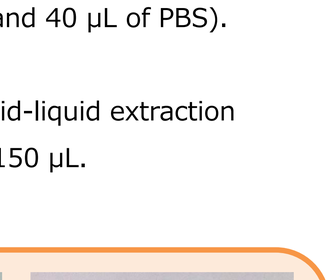

③ Removing upper patty


④ Making a slit with a knife


⑤ Snapped off at the slit


⑥ Equipped to a Capillary Micropipette


⑦ Ejecting plasma into a microtube


⑧ Weighing to convert weight to volume

Results

1. Method validation parameter - Precision and accuracy

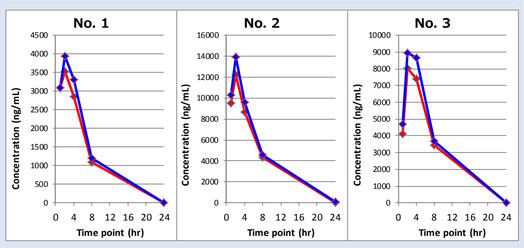
No.	LLQC (1 ng/mL)	LQC (3 ng/mL)	MQC (50 ng/mL)	HQC (800 ng/mL)	Dilution QC (2000 ng/mL) 10-fold	Dilution QC (2000 ng/mL) 100-fold
1	1.03	2.88	51.2	815	2200	2220
2	1.04	2.90	51.6	804	2150	2280
3	0.994	2.83	51.3	804	2190	2370
4	1.02	2.85	51.1	817	2200	2260
5	1.00	3.08	51.3	803	2160	2270
Mean (ng/mL)	1.02	2.91	51.3	809	2180	2280
SD	0.02	0.10	0.2	7	20	60
CV (%)	2.0	3.4	0.4	0.9	0.9	2.6
Accuracy (%)	102.0	97.0	102.6	101.1	109.0	114.0

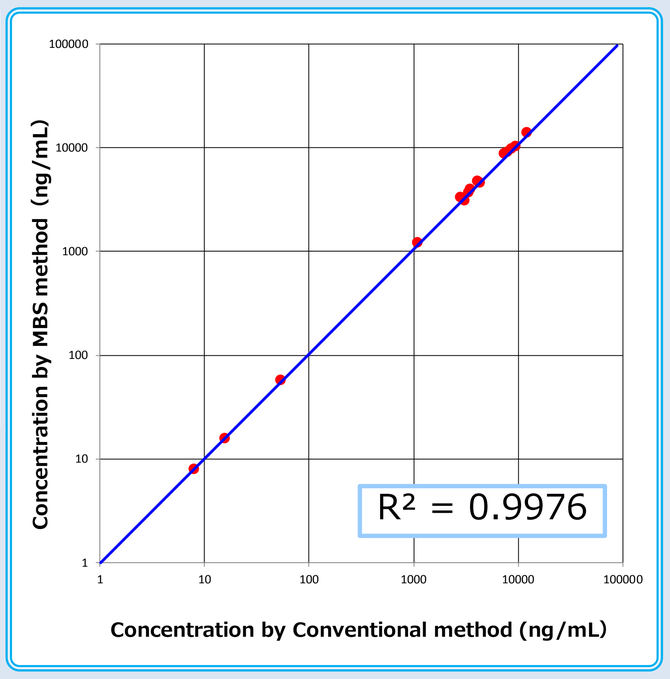
- Validation parameters met criteria that are established in the validation guideline*.

*: Guideline on Bioanalytical Method Validation in Pharmaceutical Development (Notification No. 0711-1 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare, dated July 11, 2013)

2. Correlation of MBS method and conventional method

Animal No.	Time point (hr)	Concentration by Conventional method (ng/mL)	Concentration by MBS method (ng/mL)
1	1	3100	3080
	2	3520	3930
	4	2850	3300
	8	1100	1200
	24	8.02	7.92
2	1	9530	10300
	2	12200	13900
	4	8670	9610
	8	4340	4540
	24	53.9	56.8
3	1	4120	4680
	2	8010	8960
	4	7380	8660
	8	3420	3670
	24	15.9	15.7

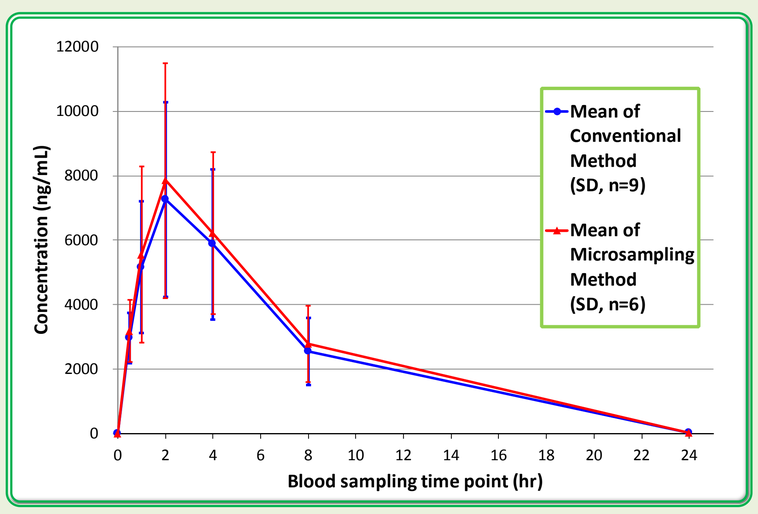




- In the blood sampling by both method from one animal at the same time, concentrations obtained by each method show good correlation at all sampling points.

3. PK parameters

Conventional method group (n=9)			
	$AUC_{0\text{-}24\ hr}$ (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)
Mean	59400	7260	2.0
SD	23300	3020	0.0
CV%	39.2	41.6	0.0
Microsampling method group (n=6)			
	$AUC_{0\text{-}24\ hr}$ (ng/mL·hr)	C_{max} (ng/mL)	T_{max} (hr)
Mean	64200	7850	2.0
SD	27000	3650	0.0
CV%	42.1	46.5	0.0



- For $AUC_{0\text{-}24\ hr}$ and C_{max}, the difference was not observed between the data from the MBS method and the data from the conventional method.

4. Hematology and blood biochemistry parameters

Group		RBC (10^4/μL)	Ht (%)	Hb (g/dL)
Non-blood collection group	mean (SD)	**720 (30)**	**44.4 (2.0)**	**15.7 (0.8)**
Conventional method group	mean (SD)	**605 (36)**	**37.6 (2.1)**	**13.0 (0.8)**
MBS method group	mean (SD)	**684 (54)**	**42.6 (2.6)**	**15.0 (0.9)**

- In a comparison between values of the MBS method and values of the conventional method, parameters that show the significant differences are shown in the above table.

- For the parameters related to the red blood cell (RBC, Ht and Hb), those parameters of conventional method group showed lower values than those of other groups.
- There were no significant differences between those values of non blood collection group and MBS method group.

Conclusion

Concentrations obtained from samples collected by both methods at the same time show good correlation. And there were no significant differences between PK parameters of both groups. Additionally, from the results of hematological parameters of red blood cell, the status of rats in the MBS group were better than rats in the conventional group.

From above results, it was considered that the MBS method was enough useful in the small animal study.