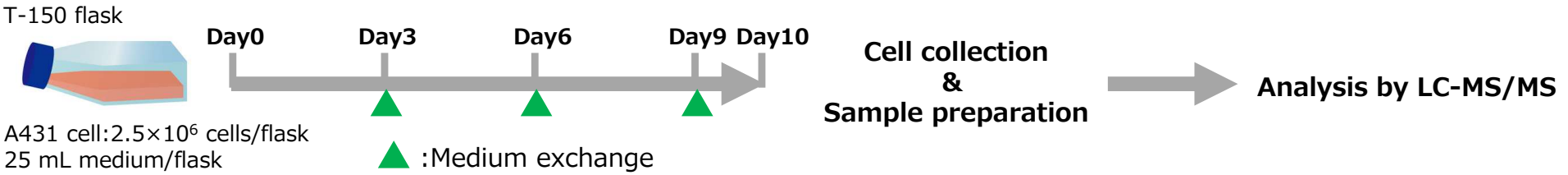
Evaluation of residual property of PG-001

Conclusion :

After culturing for **10 days** with either **10 ng/mL or 1000 ng/mL** of PG-001, the residual amount of PG-001 within the cells was **below the detection limit** for both conditions.
→ **PG-001 does not remain in the final products.**

Procedure of residual property test for PG-001



Medium Composition
- DMEM, High Glucose, GlutaMAX, Pyruvate (Thermo)
- 10 % FBS (Thermo)
- 1X MEM NEAA Solution, 100X (Gibco)
- 1:1000 Gentamicin, 50 mg/ml (Nacalai)
- 0.01% DMSO
- **PG-001: 10 ng/mL (approx. 2.07 nM) or 1000 ng/mL (approx. 207 nM)**

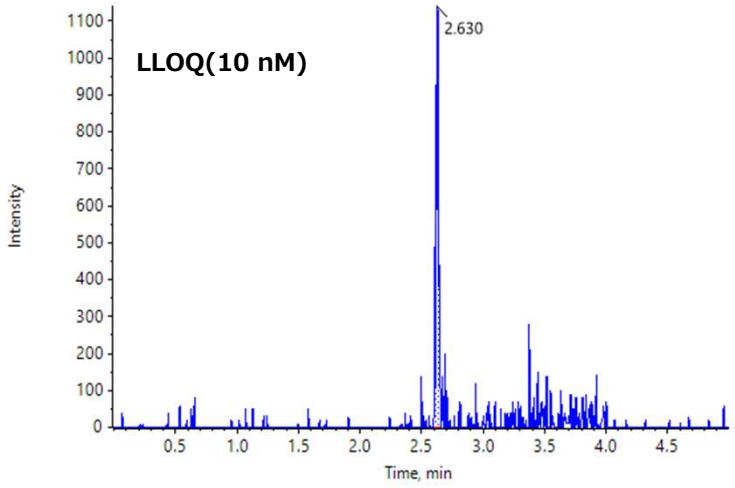
Sample Preparation & Analysis
1) Dispense into 1.0×10⁶ cells/tube
2) Cell lysis by freeze & thaw, twice
3) Addition of extraction solvent (3%, formic acid/ACN, 20 nM Veparamil) → **Internal Standard**
4) Centrifugation at 15,000xg, 10 min at 4℃
5) Take the supernatant and analysis by LC-MS/MS.

Calibration standard preparation
1) A431 cell pellet (cultured without PG-001)
2) Dispense into 1.0×10⁶ cells/tube
3) Cell lysis by freeze & thaw, twice
4) Addition of 50 uL, extraction solvent with different conc. of PG-001 **0(Blank), 10, 20, 30, 40, 50, 100, 160, and 200 nM**
5) Centrifugation at 15,000xg, 10 min at 4℃
6) Take the supernatant and analysis by LC-MS/MS

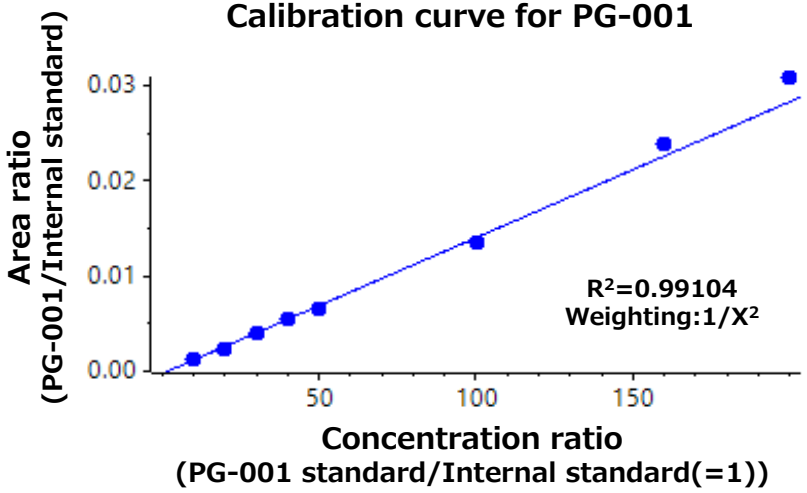
Evaluation of residual property of PG-001

Result detail

Example of chromatograms of PG-001



Calibration curve for PG-001



Lower limit of Quantification(LLOQ):

10 nM→ 2.35 ng (10 nM ×4705.29 g/mol×50 uL)

Measurement result of LC-MS/MS

10 ng/mL

Area:PG-001	Area: IS	Observed Conc.(nM)
N/A	1335384	Below LLOQ
91	1347377	Below LLOQ
79	1349176	Below LLOQ

1000 ng/mL

Area:PG-001	Area: IS	Observed Conc.(nM)
54	1352723	Below LLOQ
44	1359470	Below LLOQ
54	1353460	Below LLOQ

Calibration standard:10 nM(LLOQ)

Area:PG-001	Area: IS	Observed Conc.(nM)
1689	1310059	**10.7 nM**

✓ **PG-001 does NOT remain in cells**