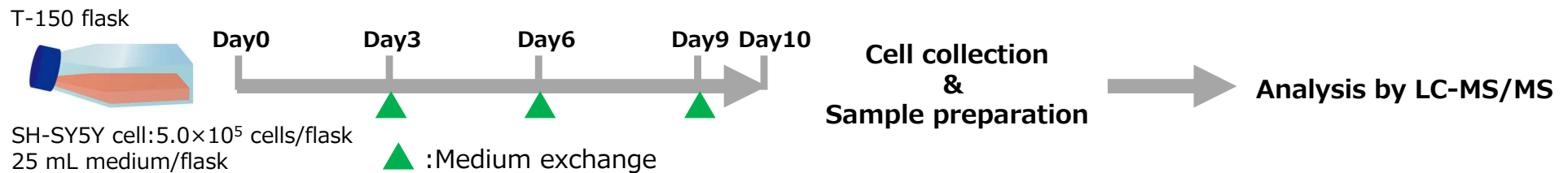


Conclusion :

After culturing for **10 days** with either **10 ng/mL** or **1000 ng/mL** of PG-001, the residual amount of PG-003 within the cells was **below the detection limit** for both conditions.
→ **PG-003 does not remain in the final products.**

Procedure of residual property test for PG-003



Medium Composition

- DMEM/F-12, HEPES (Thermo)
- 10 % FBS (Thermo)
- 1:1000 Gentamicin, 50 mg/ml (Nacalai)
- 0.01% DMSO
- **PG-003: 10 ng/mL (approx. 1.94 nM) or 1000 ng/mL (approx. 194 nM)**

Sample Preparation & Analysis

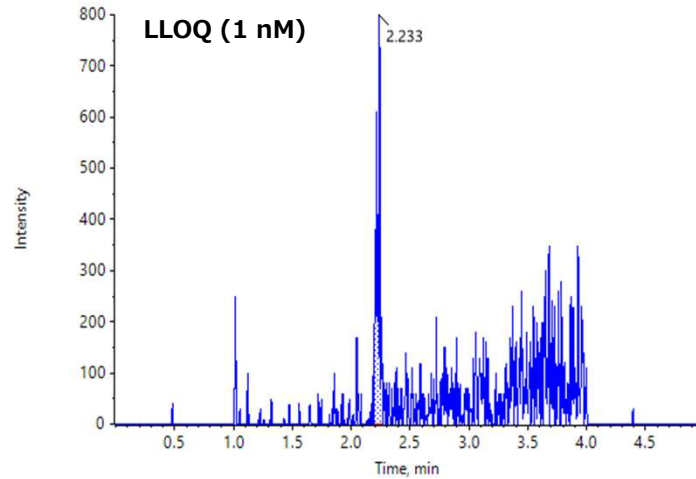
- 1) Dispense into 1.0×10^6 cells/tube
- 2) Cell lysis by freeze & thaw, twice
- 3) Addition of extraction solvent (3%, formic acid/ACN, 20 nM Veparamil)
→ **Internal Standard**
- 4) Centrifugation at 15,000xg, 10 min at 4°C
- 5) Take the supernatant and analysis by LC-MS/MS.

Calibration standard preparation

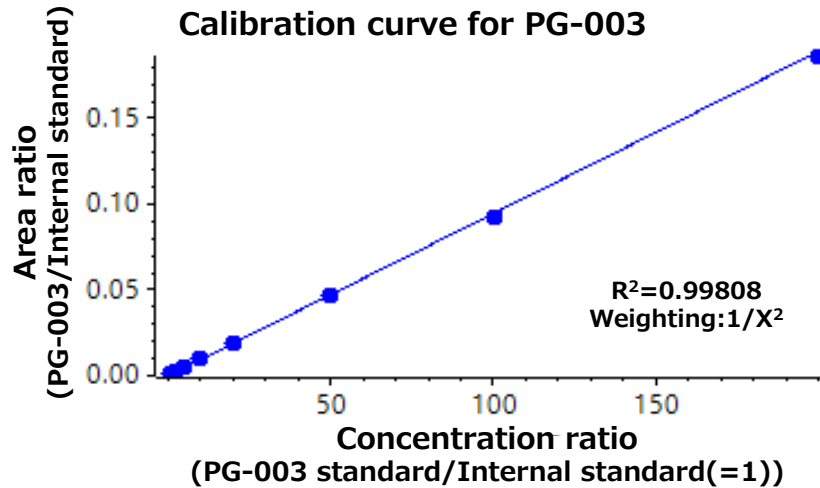
- 1) SH-SY5Y cell pellet (cultured without PG-003)
- 2) Dispense into 1.0×10^6 cells/tube
- 3) Cell lysis by freeze & thaw, twice
- 4) Addition of 50 uL, extraction solvent with different conc. of PG-001 **0(Blank), 1, 2, 5, 10, 20, 50, 100, and 200 nM**
- 5) Centrifugation at 15,000xg, 10 min at 4°C
- 6) Take the supernatant and analysis by LC-MS/MS

Result detail

Example of chromatograms of PG-003



Calibration curve for PG-003



Lower limit of Quantification(LLOQ):

1 nM → 0.25 ng (1 nM × 4911.45 g/mol × 50 uL)

Measurement result of LC-MS/MS

10 ng/mL

Area:PG-003	Area: IS	Observed Conc.(nM)
63	1316968	Below LLOQ
49	1319367	Below LLOQ
41	1330087	Below LLOQ

1000 ng/mL

Area:PG-003	Area: IS	Observed Conc.(nM)
47	1317636	Below LLOQ
271	1306060	Below LLOQ
135	1297578	Below LLOQ

Calibration standard: 1 nM(LLOQ)

Area:PG-003	Area: IS	Observed Conc.(nM)
1285	1342365	1.0 nM

✓ PG-003 does NOT remain in cells