**INTRODUCTION**

FF-10502 (F), a structural analog of nucleoside deoxycytidine (dC), is a pyrimidine nucleoside antimetabolite anticancer agent. Pyrimidine antimetabolites exert cell cycle phase-specific activity by killing cells undergoing DNA synthesis (S-phase), and blocking the progression of cells through the G1/S-phase boundary. To evaluate F incorporation into whole blood cellular DNA as a pharmacodynamic marker requires having a method to simultaneously determine F and deoxycytidine (dG) in human DNA. The key aspects of this method includes 1) achieving the LLOQ for F at 5 pg/mL; 2) one-step DNA hydrolysis procedure to reduce the process time and avoid the need for heat denature the DNA at 95°C; 3) simultaneous quantifying F and dG with the concentration difference greater than 10^4; 4) having F-incorporated DNA obtained from murine tumor tissues as an assay control on DNA hydrolysis and assay performance; and 5) independent of amount of DNA analyzed giving consistent ratio of F to dG.

**ASSAY DEVELOPMENT CONSIDERATIONS**

1. Simplify DNA hydrolysis process – one step, instead of two steps: no DNA denature step at 95°C. It was achieved by applying DNA Degradate Plus mix.
2. Simultaneous determination of F and dG with concentration difference greater than 10^4.
3. Monitor the impact of DNA hydrolysis and assay performance on F/dG ratio from batch to batch.
4. Evaluate the difference in DNA amount on F/dG ratio. Include F-DNA obtained from murine tumor tissue as a control in every batch.
5. Monitor the impact of DNA Degradate Plus and overnight incubation at 37°C on the assay performance considering STDs and QCs prepared in surrogate matrix were not subjected to DNA hydrolysis step. Two additional controls are included in each batch: Surrogate Matrix Blank with DNA hydrolysis step and QOC with DNA hydrolysis step.

**SAMPLE PROCESSING**

All DNA samples were dissolved in and diluted with DNA hydration buffer. STDs, QCs and other test samples were prepared in surrogate matrix.

**DNA INCORPORATION/PD MARKER**

F (Inhibitor of DNA synthesis)

**LC-MS/MS CONDITIONS**

- **HPLC**: Shimadzu Nexera
- **MS/MS**: AB Sciex API5000
- **Column**: HSBS T3, 2.1 x 50 mm, 1.8 µm, Waters
- **Mobile Phases**: 5 mM ammonium formate and 0.45% formic acid in water / MeOH

**RESULTS**

**Conclusions**

1. A one-step DNA hydrolysis procedure was successfully developed and applied for this method.
2. LC-MS/MS conditions enabled simultaneous quantification of two analytes with a concentration difference greater than 10^4.
3. F-incorporated DNA obtained from murine tumor tissue was applied as an assay control on DNA hydrolysis and assay performance by comparing the F/dG ratio from batch to batch.
4. The assay has successfully applied to analyze DNA samples isolated from mouse and human studies.