An LC-MS/MS Method for the Evaluation of FF-10502 Incorporation into Whole Blood Cellular DNA as a Pharmacodynamic Marker

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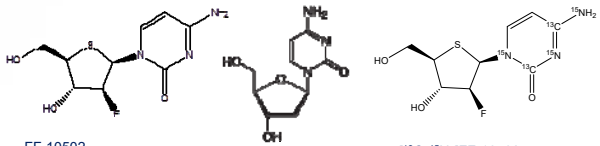
INTRODUCTION

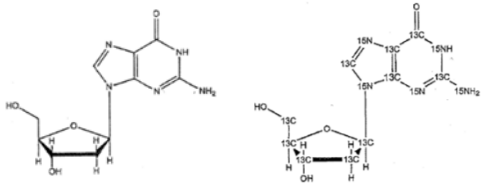
FF-10502 (F), a structural analog of nucleoside deoxycytidine (dC), is a pyrimidine nucleoside antimetabolite anticancer agent. Pyrimidine antimetabolites exert cell cycle phase-specific activity by killing cells undergoing DNA synthesis (S-phase), and blocking the progression of cells through the G1/S-phase boundary. To evaluate F incorporation into whole blood cellular DNA as a pharmacodynamic marker requires having a method to simultaneous determines F and deoxyguanosine (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 to heat denature the DNA at 95°C; 3) simultaneous quantifying F and dG with the concentration difference greater than 10^5 ; 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.



FF-10502
$C_9H_{12}FN_3O_3S$, MW: 261.27

Deoxycytidine

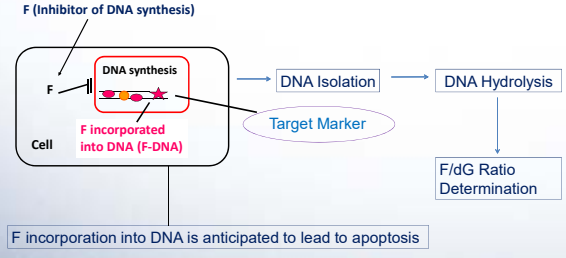
$[^{13}C_2{}^{15}N_3]$FF-10502
$C_7{}^{13}C_2H_{12}F^{15}N_3O_3S$



2'-Deoxyguanosine
$C_{10}H_{13}N_5O_4$, MW: 267.24

Deoxyguanosine-$^{13}C_{10}{}^{15}N_5$
$^{13}C_{10}H_{13}{}^{15}N_5O_4$

DNA INCORPORATION/PD MARKER



ASSAY DEVELOPMENT CONSIDERATIONS

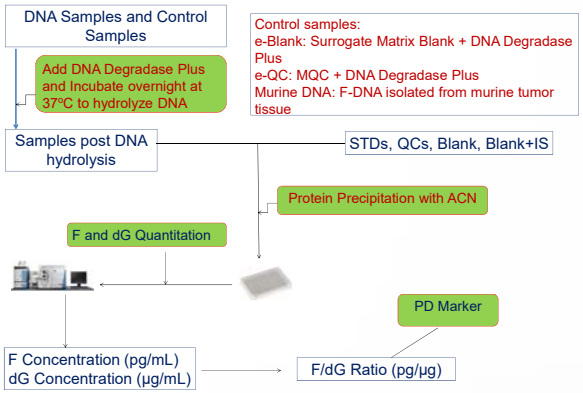
1. Simplify DNA hydrolysis process – one step, instead of two steps; no DNA denature step at 95°C. It was achieved by applying DNA Degradate Plus mix.
2. Simultaneous determination of F and dG with concentration difference greater than 10^5.
3. Monitor the impact of DNA hydrolysis and assay performance 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 Degradase 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 MQC with DNA hydrolysis step.

SMAPLE PROCESSING

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

Surrogate matrix: 10 µg/mL of each of dA, dC and T in DNA hydration buffer

DNA hydrolysis

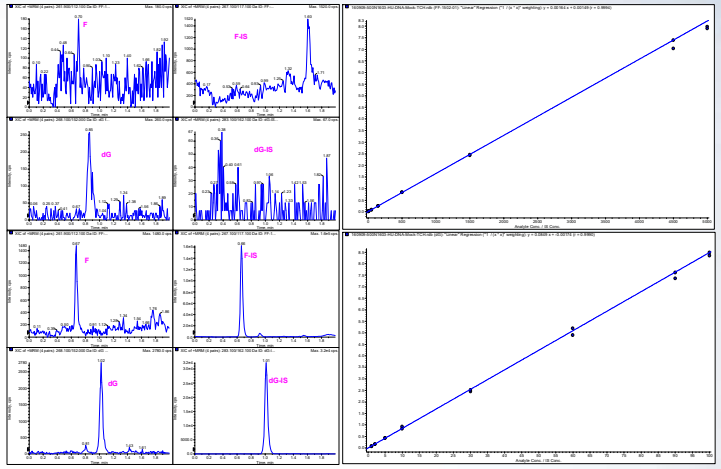


LC-MS/MS CONDITIONS

HPLC: Shimadzu Nexera
MS/MS: AB Sciex API6500
Column: HSS T3, 2.1 x 50 mm, 1.8 µm, Waters
Mobile Phases: 5 mM ammonium formate and 0.45% formic acid in water / MeOH

Compound	DE (V)	CE (eV)	CXP (V)	Transition(m/z)
F	46	19	12	261.9→112.1
F-IS	46	19	12	267.1→117.1
dG	240	50	15	268.1→152.0
dG-IS	240	50	15	283.1→162.1

RESULTS



Precision and Accuracy of the Assay

Analyte		LLOQ QC		L, M and HQC		e-QC	
		Intra-Run	Inter-Run	Intra-Run	Inter-Run	Intra-Run	Inter-Run
F	RSD	3.5-6.5	6.4	0.3-7.5	1.6-8.0	1.0-1.7	5.2
(5 – 5000 pg/mL)	%RE	-7.9-4.7	-3.4	-7.4-10.5	-2.3-3.6	-3.5-8.4	3.6
dG	RSD	1.9-12.4	10.3	1.7-7.6	3.4-5.8	3.8-6.0	4.4
(1 – 100 µg/mL)	%RE	-11.5-9.3	0.2	-5.8-6.0	-2.7-2.4	2.9-5.3	3.9

F and dG Concentrations in Murine DNA Samples

	F (pg/mL)	dG (µg/mL)	F/dG (pg/µg)	F (pg/mL)	dG (µg/mL)	F/dG (pg/µg)	Inter-Run
	Run 1, 50 µg/mL Murine DNA			Run 2, 50 µg/mL Murine DNA			F/dG (pg/µg)
Mean	416	6.81	60.9	843	12.9	65.6	65.7
RSD	13.6	4.5	10.3	2.3	2.7	3.5	8.8
	Run 3, 50 µg/mL Murine DNA			Run 4, 20 µg/mL Murine DNA			
Mean	848	12.5	68.6	354	5.25	67.5	
RSD	2.0	10.8	12.1	7.1	7.9	6.2	

F and dG Concentrations in Mouse Blood DNA Samples

Sample ID	F (pg/mL)	dG (µg/mL)	F/dG (pg/µg)
5021A	BQL	1.55	NA
5022A	BQL	1.68	NA
5023A	BQL	2.18	NA
2401A	105	2.23	47.1
2402A	63.7	1.41	45.2
2403A	66.9	1.54	43.4
4801A	79.6	1.33	59.8
4802A	81.5	1.48	55.1
4803A	77.4	1.30	59.5

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 10^5.
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.