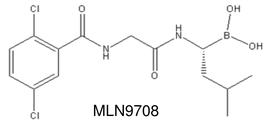
Development of a UPLC-MS/MS Method for the Determination of MLN9708 and its Enantiomer from Human Plasma

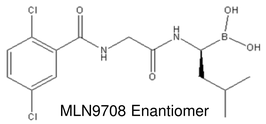
Lan Li[a], Xiaohan Cai[a], Tracey Paxson[a], Yuan-Shek Chen[a], Ben Hsu[a]; Martin Paton[b], Mark Qian[b]
[a]QPS, LLC; [b]Millennium: The Takeda Oncology Company

INTRODUCTION

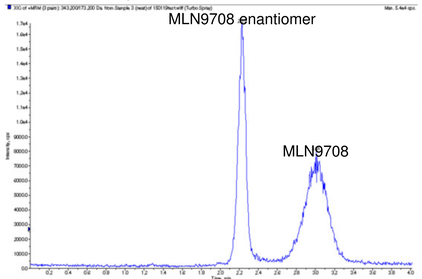
The purpose of this work was to develop a chirally selective method for the quantitative analysis of MLN9708 (with a boronic acid moiety at its chiral center) and its enantiomer from K_2EDTA human plasma. Due to the inadequate retention of MLN9708 and its enantiomer on reverse phase columns, the chiral separation was achieved on a Chiralpak® IC™ column with a normal phase retention mechanism in neat analyte solutions. However, the condition was not suitable for the extracts from human plasma samples due to the observation of highly noisy chromatography baselines and shifted retention time. This issue associated with the extracted samples cannot be resolved by modifying the method despite several attempts. Besides, due to the broad analyte peaks, achieving the target LLOQ could be challenging even if a compatible extraction method could be developed. To overcome the challenges, the approach is to convert MLN9708 and its enantiomer with a chiral derivatization reagent (1S,2S,3R,5S)-(+)-pinanediol into diastereomers and have them chromatographically separated by UPLC achiral column. Indeed, an LC condition with Acquity UPLC® BEH Phenyl column gave not only baseline separation but also clean baseline. In addition, better sensitivity was achieved in this approach due to much narrower peaks. The method was successfully applied to the analysis of MLN9708 and its enantiomer from human plasma samples.



MLN9708



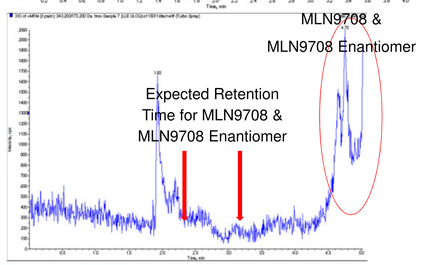
MLN9708 Enantiomer



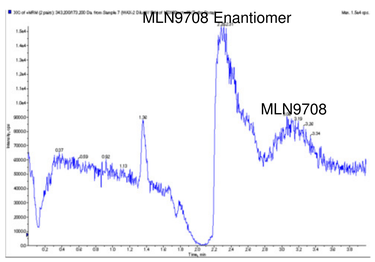
Column:
Chiralpak IC™, 2.0X150 mm, 5 µm

Mobile Phases:
A: Heptane
B:Isopropanol:Ethanol@50:50 (V:V)

Sample:
MLN9708/MLN9708 enantiomer
500/500 ng/mL in Heptane:Isopropanol:Ethanol @ 80:10:10 (V:V:V)



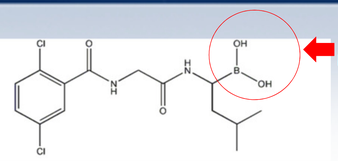
Sample:
ULOQ level human plasma sample extracted by LLE (validated by the achiral assay) and re-dissolved in IPA



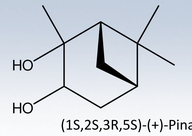
Sample:
ULOQ level human plasma sample extracted by SPE (WAX) and re-dissolved in IPA

METHODS

The quantification utilized $^{13}C_9$-MLN9708 as the internal standard. The plasma samples were extracted by solid-phase extraction (SPE) with Waters Oasis® WAX 96-Well SPE Plates (30 mg). The extracted samples were re-dissolved in (1S,2S,3R,5S)-(+)-pinanediol solution [2 mg/mL in acetonitrile (ACN) : water : formic acid (FA) @ 20:80:0.1, V:V:V] and incubated at 37° C for approximately 1 hour. The samples were then injected onto an Acquity UPLC® BEH Phenyl column. MLN9708 and its enantiomer were eluted with a mixture of 10 mM ammonium formate (pH 3.0), ACN, and FA at a flow rate of 0.4 mL/min,. The pinanediol derivatized MLN9708 and its enantiomer were detected by an AB Sciex API 6500 mass spectrometer.



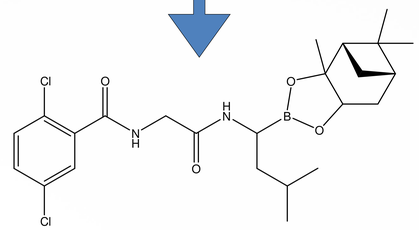
Reactive to *cis*-diols Under **Neutral or Basic** Conditions

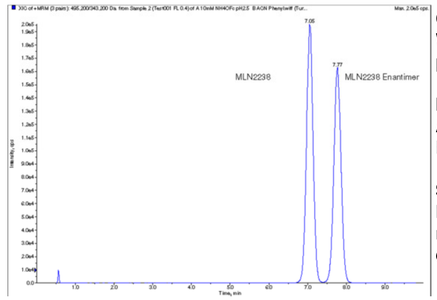


(1S,2S,3R,5S)-(+)-Pinanedio

In ACN w/ 1% NH_4OH
37° C, 1 hr

~85% Yield





Column:
Waters Acquity BEH Phenyl, 2.0X50 mm, 1.7 µm

Mobile Phases:
A: 10 mM Ammonium Formate, pH 3.0
B: Acetonitrile:Formic acid @ 100:0.1 (V:V)

Sample:
MLN9708/MLN9708 enantiomer, 500/500 ng/mL in Acetonitrile:Water:Formic Acid @20:80:0.1 (V:V:V)

Plate Activated w/ MeOH
Equilibrated w/ Water
↓
Sample Loading
↓
Plate Washed w/ H_2O
↓
Sample Eluted w/
ACN:NH_4OH@100:1 (V:V)
↓
Adding Derivatization Agent
37° C 1 hr
↓
Dry Down @ 40° C
↓
Reconstitution in ACN:H_2O
@20:80 (V:V)

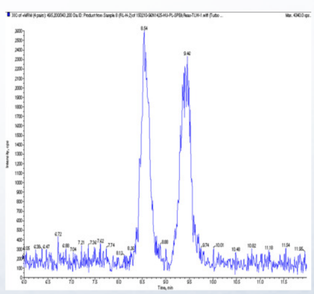
Low Analyte Signal (<10% of Theoretical)!

- Low recovery (~5%) with ACN:NH_4OH@100:1 (V:V) elution
- Improved recovery (~50%) with MeOH:NH_4OH@100:1 (V:V) elution, but with unstable derivatization product
- Can not be re-dissolved in ACN:NH_4OH@100:1 (V:V) after dry-down
- Best reconstitution solvent (ACN:Water:**FA**) dose not appeare to be favored by the derivatization reaction per literatures

Would the Reaction Happen under Acidic Condition?

Derivatize in ACN : Water : FA @ 20:80:0.1 (V:V:V), 37° C, 1 hr

~90% Yield



Chromatogram of an Extracted LLOQ Level Sample

RESULTS

For both analytes, the method is capable of achieving a lower limit of quantification (LLOQ) of 0.5 ng/mL, the same LLOQ acheived by an achiral assay of MLN9708. The calibration range of the method was 0.5-500 ng/mL for both analytes. The linearity, accuracy, and precision were demonstrated by a successful qualification run. The methods also demonstrated good selectivity and minimum matrix effect. The qualified method was utilized for the analysis of non-GLP samples, examining the existence of MLN9708 enantiomer from subjects dosed with MLN9708.

MLN9708 Std	0.5 (ng/mL)	1 (ng/mL)	5 (ng/mL)	15 (ng/mL)	50 (ng/mL)	150 (ng/mL)	450 (ng/mL)	500 (ng/mL)
Calculated Conc., ng/mL	0.524	0.992	5.13	15.4	48.6	154	453	516
	0.482	0.977	5.08	14.9	47.1	148	439	507
	0.533	0.962	5.22	15.1	50.3	156	450	509
	0.482	0.963	5.09	14.8	48.7	150	439	488
Mean	0.505	0.974	5.13	15.1	48.7	152	445	505
S.D.	0.0271	0.0141	0.0638	0.265	1.31	3.65	7.32	12.0
%CV	5.4	1.4	1.2	1.8	2.7	2.4	1.6	2.4
%RE	1.0	-2.6	2.6	0.7	-2.6	1.3	-1.1	1.0

MLN9708 Enantiomer Std	0.5 (ng/mL)	1 (ng/mL)	5 (ng/mL)	15 (ng/mL)	50 (ng/mL)	150 (ng/mL)	450 (ng/mL)	500 (ng/mL)
Calculated Conc., ng/mL	0.477	1.02	5.28	14.7	45.2	158	460	530
	0.512	1.01	5.24	14.4	42.8	150	457	518
	0.544	0.965	4.94	15.0	51.2	155	447	518
	0.467	0.993	4.96	14.9	48.7	151	454	485
Mean	0.500	0.997	5.11	14.8	47.0	154	455	513
S.D.	0.0351	0.0241	0.18	0.265	3.72	3.7	5.57	19.3
%CV	7.0	2.4	3.5	1.8	7.9	2.4	1.2	3.8
%RE	0.0	-0.3	2.2	-1.3	-6.0	2.7	1.1	2.6

MLN9708 QC	LLOQ (0.5 ng/mL)	LQC (1.5 ng/mL)	LMQC (40 ng/mL)	MQC (200 ng/mL)	HQC (400 ng/mL)
Calculated Conc., ng/mL	0.502	1.47	40.0	198	411
	0.437	1.49	40.9	193	402
	0.484	1.50	39.2	201	412
Mean	0.474	1.49	40.0	197	408
SD	0.0336	0.0153	0.850	4.04	5.51
%CV	7.1	1.0	2.1	2.1	1.4
%RE	-5.2	-0.7	0.0	-1.5	2.0

MLN9708 Enantiomer QC	LLOQ (0.5 ng/mL)	LQC (1.5 ng/mL)	MQC1 (40 ng/mL)	MQC2 (200 ng/mL)	HQC (400 ng/mL)
Calculated Conc., ng/mL	0.554	1.38	38.6	200	432
	0.477	1.43	39.9	197	417
	0.525	1.47	37.7	205	426
Mean	0.519	1.43	38.7	201	425
SD	0.0389	0.0451	1.11	4.04	7.55
%CV	7.5	3.2	2.9	2.0	1.8
%RE	3.8	-4.7	-3.2	0.5	6.3

NOVEL ASPECT

Identified an excellent agent for the derivatization of the boronic acid moiety to overcome the challenges on chromatography, chiral seperation and needed sensitivity at the same time.