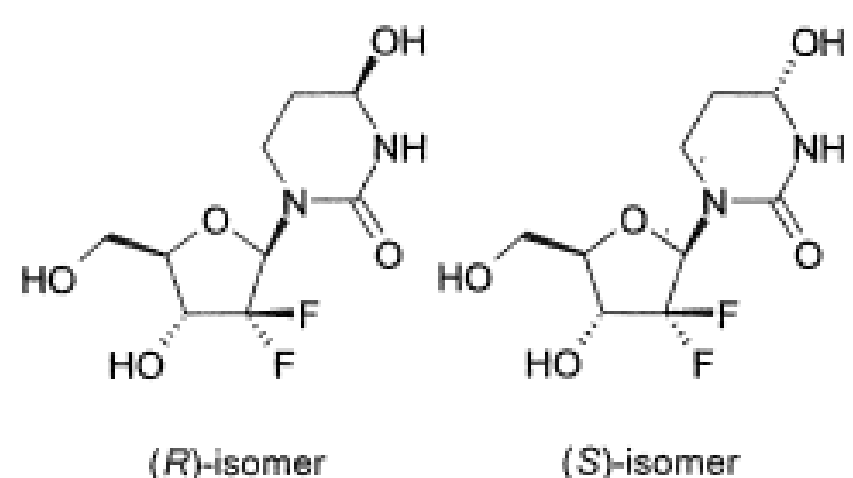


Development and Validation of an LC-MS/MS Method for the Simultaneous Quantitation of Cedazuridine (E7727), Epimer of Cedazuridine and Decitabine in THU-stabilized K2EDTA Human Plasma

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Purpose

Cedazuridine (E7727) is a novel cytidine deaminase inhibitor that inhibits the in vivo degradation pathway of β -decitabine (decitabine) when administered orally in combination with decitabine (known as ASTX727) in clinical trials.



E7727 (R) and E7727 Epimer (S)

- E7727 partially converts to its epimer in-vivo (at acidic pH).
- A sensitive and robust LC-MS/MS method is needed to support PK evaluation of E7727, E7727-epimer and decitabine in clinical studies.
- An LC-MS/MS 3-in-1 method (single extraction) for simultaneous quantitation of E7727, E7727-epimer and decitabine in human plasma have been developed and validated.

Method Development

Challenges	Resolution
Instability of decitabine	Spiked with THU to stabilize analytes in plasma/whole blood (concentration of THU 2.8 $\mu\text{g/mL}$ in plasma/blood)
Interference of α -decitabine to β -decitabine	Normal-Phase column to achieve separation
Separation of E7727-epimer from E7727	A reverse phase column was selected and achieve baseline separation
Sensitivity	0.5/5/3.18 – 200/2000/1270 ng/mL for decitabine/E7727/E7727-Epimer achieved

Method and Results

- Protein precipitation (PPT) was used to extract decitabine, E7727 and E7727-epimer from THU-stabilized human plasma samples (50 μL) followed by LC-MS/MS analysis for E7727, E7727-epimer and decitabine with two different HPLC columns (reverse phase for E7727 and E7727-epimer and normal phase for decitabine) under different conditions.
- HPLC-MS/MS conditions for E7727 and E7727-epimer

Shimadzu LC-30AC pumps/Sciex API5000, or Qtrap5500 Column: Phenomenex, Gemini C6-Phenyl, 50 x 4.6 mm, 3 μm Mobile Phase A: 5 mM Ammonium formate in H_2O Mobile Phase B: 5 mM Ammonium formate in ACN/MeOH, 1:1 v/v Interface: TurbolonSpray (ESI), Positive MRM channels: E7727 and E7727 epimer: 269.3 \rightarrow 99.0 Deutero E7727 and E7727 epimer (IS): 273.3 \rightarrow 103.0	HPLC Gradient		
	Time/min	%B	Flow Rate (mL/min)
	0.01	5	0.75
	2.40	35	0.75
	2.70	100	0.75
	3.20	100	0.75
	5.60	100	2.00
	5.70	5	0.75
	7.30	Stop	

- HPLC-MS/MS conditions for decitabine

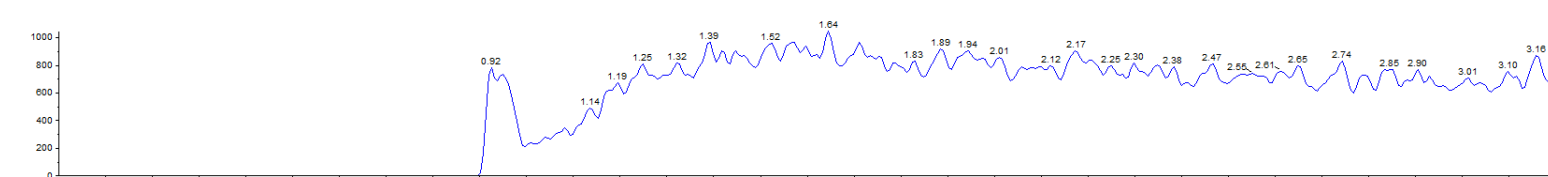
Shimadzu UFLC/LC-20AD pumps/Sciex API4000, 5000, or Qtrap5500 Column: Waters Nova-Pak Silica, 150 x 3.9 mm, 4 μm Mobile phase A: 5mM Ammonium formate in H_2O Mobile phase B: 5mM Ammonium formate in ACN: H_2O , 98:2 v/v Interface: TurbolonSpray (ESI), Positive MRM channels: Decitabine: 229.0 \rightarrow 113.1 Decitabine- d_3 (IS): 232.0 \rightarrow 113.1	HPLC Gradient		
	Time/min	%B	Flow Rate (mL/min)
	0.00	97	1.4
	4.20	97	1.4
	4.70	2	1.4
	8.70	2	1.4
	8.80	97	1.4
	10.80	Stop	

Results (cont.)

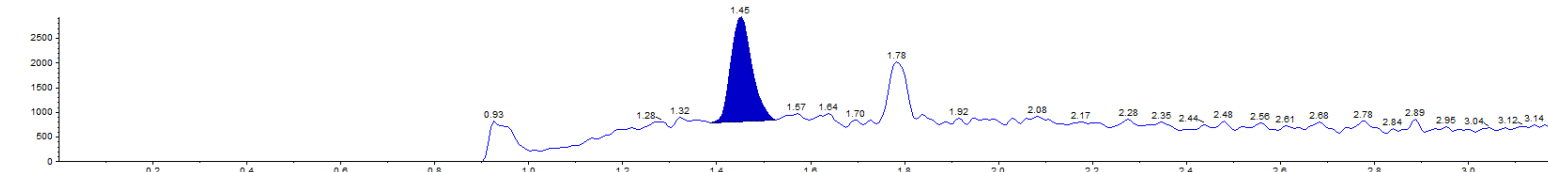
- Precision and Accuracy (Decitabine, E7727 and Epimer)

		QC LLOQ	Intra-run QC Low	Intra-run QC Mid	Intra-run QC High
		0.500 ng/mL	1.50 ng/mL	15.0 ng/mL	150 ng/mL
Decitabine	Inter-run Mean	0.494	1.45	15	150
	Inter-run SD (n=18)	0.014	0.046	0.441	3.89
	Inter-run %CV	2.7	3.1	2.9	2.6
	Inter-run %Bias	-1.2	-3.3	0	0
E7727	Inter-run Mean	4.94	14.7	151	1500
	Inter-run SD (n=18)	0.254	0.564	3.16	31.1
	Inter-run %CV	5.1	3.8	2.1	2.1
	Inter-run %Bias	-1.2	-2	0.7	0
E7727 Epimer	Inter-run Mean	3.32	9.27	96.8	944
	Inter-run SD (n=18)	0.234	0.475	5.73	36.1
	Inter-run %CV	7	5.1	5.9	3.8
	Inter-run %Bias	4.4	-2.7	1.6	-0.9

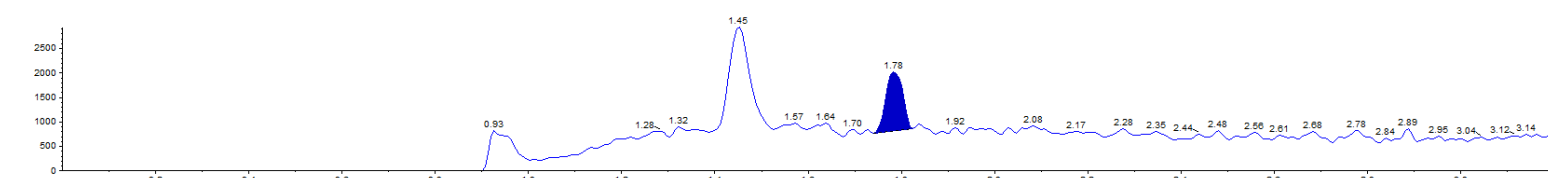
Double Blank E7727 and Epimer



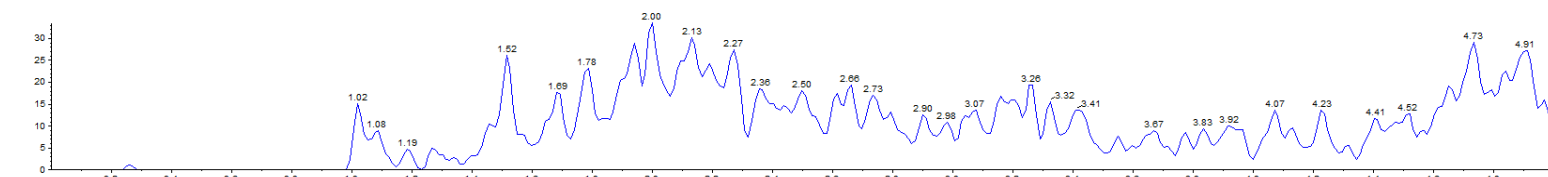
LLOQ E7727



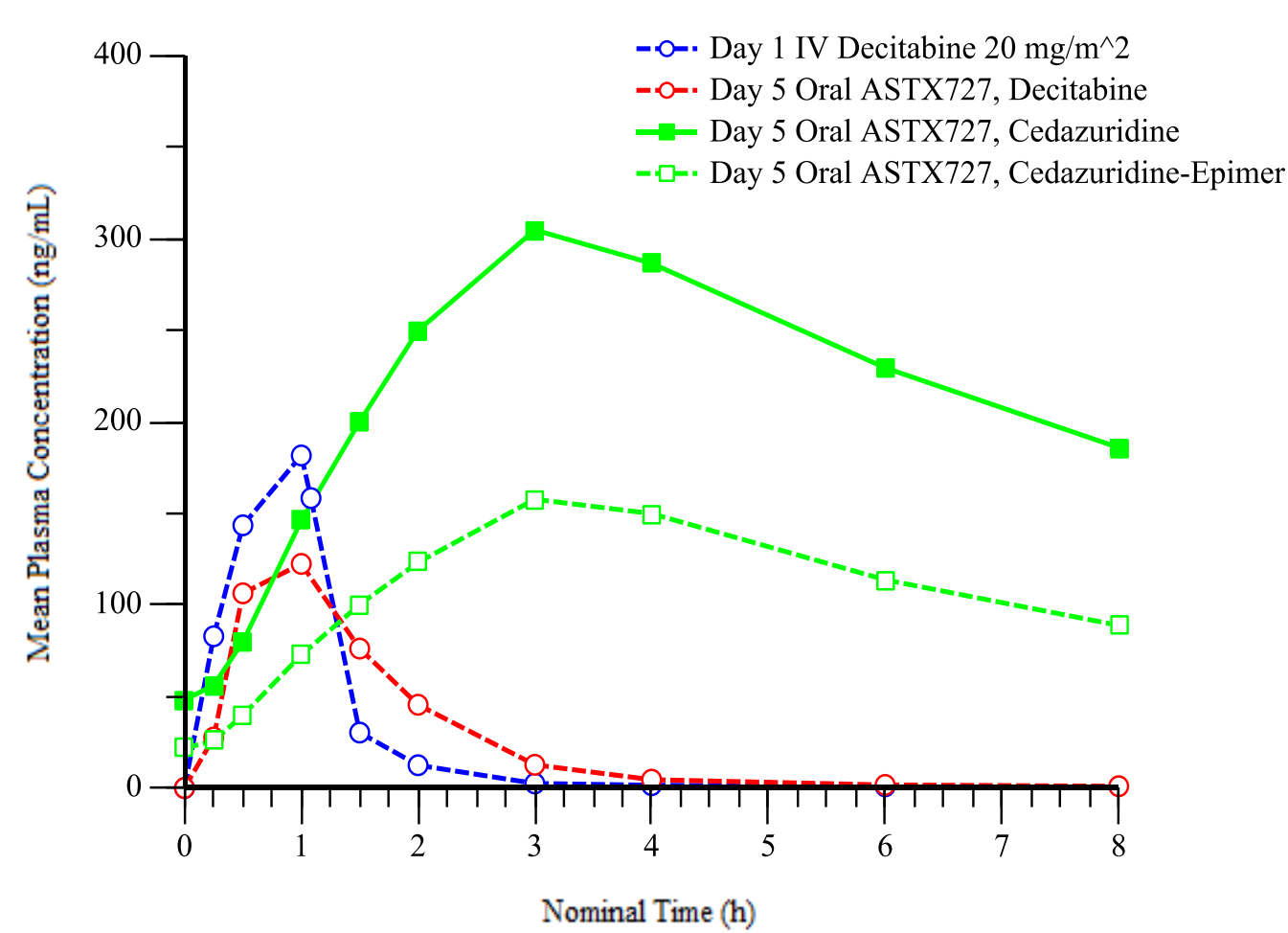
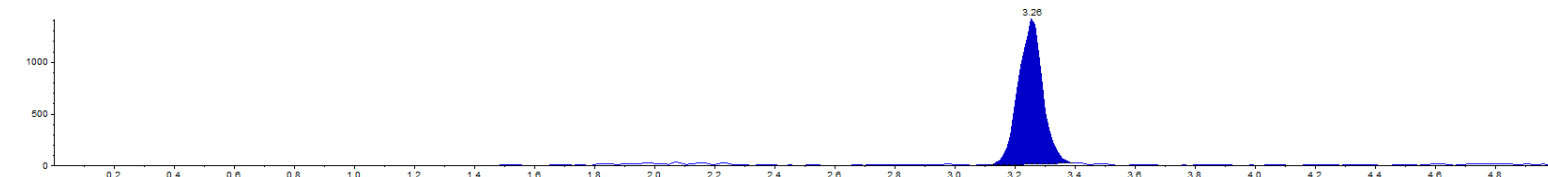
LLOQ E7727 Epimer



Double Blank Decitabine



LLOQ Decitabine



Conclusion

- A robust, high throughput method for the simultaneous determination of decitabine, E7727 and E7727-epimer in human plasma by LC-MS/MS using simple protein precipitation procedure was successfully developed and validated according to FDA and EMA guidelines and used for several clinical studies to evaluate the PK of decitabine, E7727, and E7727-epimer in human plasma.

