A Sensitive and Efficient LC-MS/MS Method for Creatinine-d_5 Analysis in *In Vitro* OCT2 Inhibition Assay

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Abstract

In vitro transporter inhibition studies are designed to determine if the investigational drug is an inhibitor of a specific transporter. The Organic Cation Transporter 2 (OCT2), expressed predominantly in renal tubular epithelial cells, primarily transports hydrophilic, low molecular weight organic cations. Creatinine, a validated substrate of OCT2, serves as an *in vitro* indicator of OCT2 activity.
However, as an endogenous substance with high polarity, creatinine poses challenges due to its difficult retention in reverse phase chromatography and high baseline in the matrix. To address these issues, hydrophilic interaction chromatography was employed to ensure good and robust retention, and creatinine-d_5 was used as the substrate instead of unlabeled creatinine to avoid endogenous interference in the matrix. This approach not only facilitated efficient and rapid analysis but also achieved a lower limit of quantitation (LLOQ) of 20 pM[1], which is four times lower than those reported in the current literature.

Creatinine

Creatinine-d_5

Figure 1. The chemical structure of creatinine and creatinine-d_5

Objective

To accurately evaluate the inhibitory activity of *in vitro* OCT2 transporter, creatinine-d_5 was used instead of creatinine. This approach eliminates the interference caused by endogenous creatinine and enables the establishment of a novel and efficient LC-MS/MS method.

Method

➢ Sample Preparation

The OCT2 cells were seeded at a density of 50,000 cells/well and grown for 24 h. Creatinine-d_5 of different concentrations was added to the well, at the end of the incubation, the buffer was removed. All the samples were lysed using 100 µL of acetonitrile/methanol (95/5, v:v). After mixing, the cell lysate was transferred to a 96-well plate and diluted with an appropriate ratio of transport buffer. The mixture was centrifuged at 4000 rpm for 10 minutes. Then, an aliquot of 100 µL of supernatant was transferred to a 96-well plate, and analyzed using LC-MS/MS.

➢ LC-MS/MS Method

Table 1. LC condition and MS parameters for creatinine-d_5 in OCT2 cell lysate

LC-MS/MS Condition	
LC System	Waters ACQUITY UPLC
Mass Spectrometer	SCIEX Triple Quad 6500 Plus
Column	Atlantis Premier BEH Z-HILIC 1.7 µm 2.1 * 50 mm
Mobile Phase A	0.1% Formic acid and 10 mM NH_4OAc in Water/Acetonitrile (v/v, 95: 5)
Mobile Phase B	0.1% Formic acid and 10 mM NH_4OAc in Water/Acetonitrile (v/v, 5: 95)
MS Transition (*m/z*)	119.1 / 49.1
Run Time	2 min

Results

➢ LC Condition Optimization

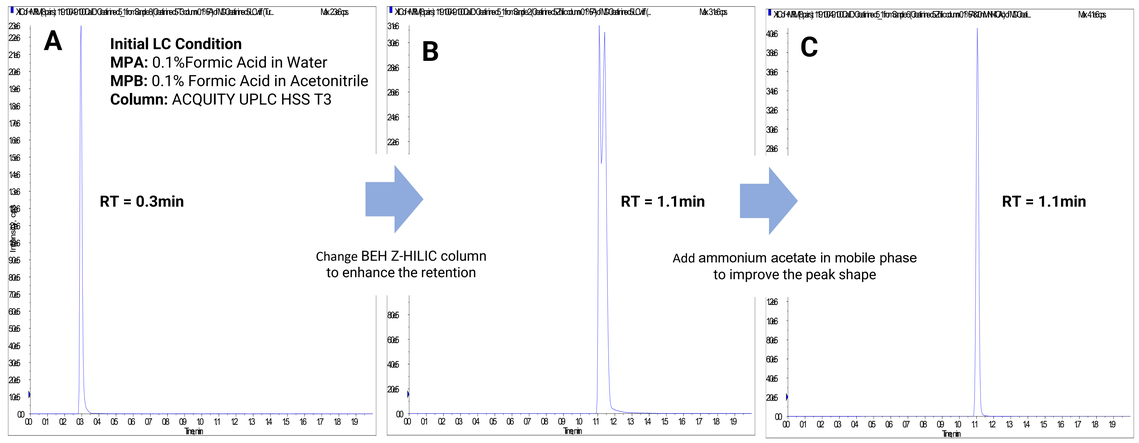

Figure 2. The chromatograms of creatinine-d_5 in different LC conditions
(A): Mobile phase: mixture of formic acid, water and acetonitrile, column: ACQUITY UPLC HSS T3
(B): Mobile phase: mixture of formic acid, water and acetonitrile, column: Atlantis Premier BEH Z-HILIC
(C): Mobile phase: mixture of NH4OAc, formic acid, water and acetonitrile, column: Atlantis Premier BEH Z-HILIC

➢ Selectivity, Sensitivity and Linearity

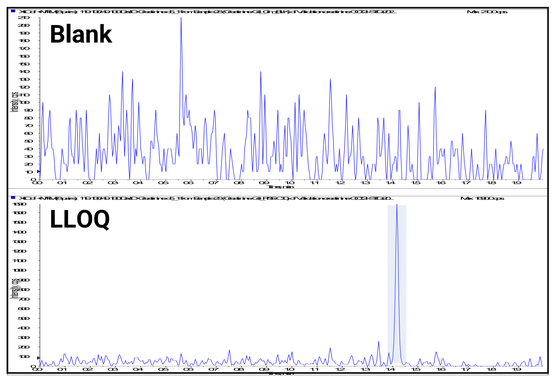

Figure 3. Chromatograms of blank and LLOQ for creatinine-d_5

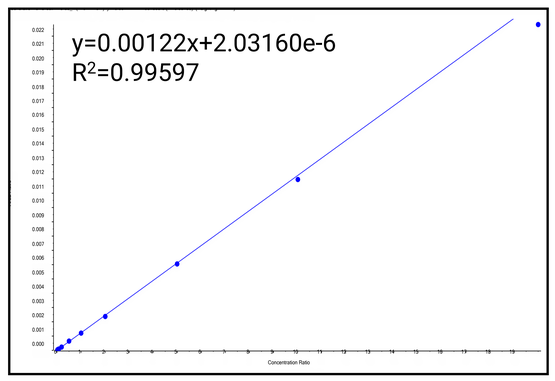

Figure 4. Calibration curve of creatinine-d_5 in OCT2 cell lysate (20-20,000 pM)

➢ Precision and Accuracy

Table 2. Precision and accuracy of quality control samples for creatinine-d_5

Analytical Run	LLOQ (20 pM)	%Bias	LQC (60 pM)	%Bias	MQC (1,000 pM)	%Bias	HQC (16,000 pM)	%Bias
Creatinine-d5	18.61	-6.95	57.84	-3.59	975.86	-2.41	15,274.43	-4.53
	18.92	-5.41	56.28	-6.20	920.25	-7.97	14,367.68	-10.20
	18.55	-7.26	51.14	-14.77	906.43	-9.36	15,256.96	-4.64
	18.55	-7.25	58.81	-1.98	1,041.19	4.12	14,230.20	-11.06
	19.79	-1.07	63.31	5.52	1,055.78	5.58	14,866.35	-7.09
	17.21	-13.97	57.26	-4.57	925.69	-7.43	14,470.51	-9.56
Within-run Mean	18.60		57.44		970.87		14,744.35	
Within-run SD	0.83		3.94		64.71		456.04	
Within-run %CV	4.47		6.86		6.66		3.09	
Within-run %Bias	-6.98		-4.27		-2.91		-7.85	
n	6		6		6		6	

Within-run %CV must be less than or equal 15%, except for LLOQ, which was 20%.
Within-run %Bias must be within ±15%, except for LLOQ, which was ±20%.

Conclusion

- The creatinine-d_5 was selected as an *in vitro* indicator of OCT2 activity, successfully avoiding the influence of endogenous background.
- A sensitive and efficient LC-MS/MS method was developed for quantifying creatinine-d_5 in OCT2 inhibition assays. Using the 2-minute analytical method, the achieved LLOQ was 20 pM, which was four times lower than previously reported values in the literature.

References

[1] Hong Shen, Tongtong Liu et al. Characterization of Organic Anion Transporter 2 (SLC22A7): A Highly Efficient Transporter for Creatinine and Species-Dependent Renal Tubular Expression. Drug Metabolism Disposition. 2015, 43, 984–993.

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