

Title: Is *N*1-methylnicotinamide a good organic cation transporter 2 (OCT2) biomarker?

Authors: Anoud Sameer Ailabouni¹, Gautam Vijaywargi¹, Sandhya Subash¹, Dilip Kumar Singh¹, Zsuzsanna Gaborik², and Bhagwat Prasad^{1,*}

Affiliation:

1. Department of Pharmaceutical Sciences, Washington State University, Spokane, WA
2. Charles River Laboratories, Solvo Biotechnology, Hungary

***Corresponding author:** Bhagwat Prasad, Ph.D., Department of Pharmaceutical Sciences, Washington State University, Spokane, WA 99202, USA. Phone: +1-509-358-7739. Fax: +1 509-368-6561. Email: bhagwat.prasad@wsu.edu

Table S1. Liquid chromatography (LC) method details for sample analysis

LC Method and Parameters			
		Parameters	
LC	M-class Waters UPLC		
MS	Waters Xevo TQ-XS		
Column	Acquity UPLC® HSS T3 (1.8 μm, 1x100 mm)		
Mobile phase A	0.1% Formic acid in water		
Mobile phase B	0.1% Formic acid in acetonitrile		
Flow rate (μL/min)	50		
Column temperature (°C)	40		
Injection volume (μL)	1		
LC gradient program (I)			
Time (min)	%A	%B	Analytes of interest: <ul style="list-style-type: none">• Metformin• N1-Methylnicotinamide
0.00	95	5	
1.00	95	5	
2.50	45	55	
4.20	32	68	
5.20	10	90	
8.00	10	90	
8.50	95	5	
11.50	95	5	
LC gradient program (II)			
Time (min)	%A	%B	Analytes of interest: <ul style="list-style-type: none">• 4-Oxo-carbazeran
0.00	95	5	
1.00	95	5	
2.50	45	55	
4.20	32	68	
5.20	10	90	
8.00	10	90	
8.50	95	5	
11.50	95	5	

Analytes of interest:

- Metformin
- N1-Methylnicotinamide

Analytes of interest:

- 4-Oxo-carbazeran

Table S2. Multiple reaction monitoring (MRM) method details for analysis of metformin, *N*1-methylnicotinamide (NMN), 4-oxo-carbazeran (4-oxo-CBZ), and internal standards.

MS method (Electrospray ionization (ESI), Positive mode)					
Compounds	Retention time (min)	Precursor <i>m/z</i>	Product <i>m/z</i>	Cone voltage (CV)	Collision energy (CE)
Metformin	1.9	130.1	60.1	25	20
		130.1	70.1	22	17
		130.1	88.1	20	15
Metformin-d6	1.9	136.1	77.1	20	20
		136.1	94.1	20	15
<i>N</i> 1-methylnicotinamide (NMN)	1.9	137.1	94.1	25	15
<i>N</i> 1-methylnicotinamide-d3 (NMN-d3)	1.9	140.1	97.1	25	15
4-Oxo-carbazeran (4-oxo-CBZ)	5.3	377.3	288.1		