Development of a sensitive and high-throughput UPLC-MS/MS method for the quantification of 1-methylnicotinamide in human serum and urine

Raymond E. West III and Thomas D. Nolin
University of Pittsburgh School of Pharmacy, Pittsburgh, PA 15261

University of Pittsburgh School of Pharmacy

INTRODUCTION

- Renal excretion of N^1-Methylnicotinamide (NMN), a metabolite of nicotinamide, is used as a biomarker of niacin status.
- NMN has been investigated as a phenotypic probe for several kidney transporters, including multidrug and toxin extrusion proteins (MATEs) and organic cation transporters (OCTs).
- Using NMN as an endogenous probe is advantageous over exogenously administered compounds.
- A simplified UPLC-MS/MS assay was developed for the quantitative determination of NMN in human serum and urine and validated using the FDA Guidance for Bioanalytical Method Validation.
- NMN concentrations were measured in samples from patients with chronic kidney disease to demonstrate application the method.

OBJECTIVE

- To develop and validate a method for the quantification of NMN in human samples.

METHODS

- Serum and urine samples (50µL) both underwent protein precipitation with a methanol-internal standard solution.
- Quality controls were prepared in stripped serum or synthetic urine.
- The separation of the samples was performed using an Acquity BEH Amide (2.1 mm x 50 mm, 1.7 µm) column with a BEH Amide 1.7 µm VanGuard Pre-Column.
- The mobile phase consisted of water with 0.1% formic acid (solvent A) and acetonitrile (solvent B).
- An isocratic elution at a flow rate of 0.4 mL/min was run on an Acquity UPLC I-class (Waters) with a total run time of 2 minutes.
- The analyte was detected in positive ion mode with selected reaction monitoring (SRM) using a heated electrospray ionization (HESI) source for ionization on a triple quadrupole mass spectrometer (Thermo Scientific).

Figure 1. The chemical structures of the analyte and internal standard.

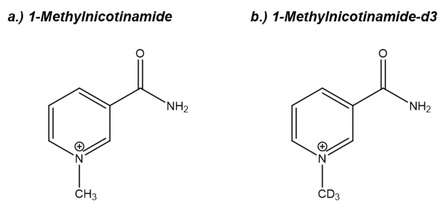


Table 1. SRM parameters for the analyte and internal standard.

Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Collision Energy (V)	Tube Lens (V)
NMN	137.0	94.1	24	65
NMN-*d3*	140.0	97.2	30	86

RESULTS

Table 2. Results of the assay validation including LLOQ and linear range. The slope, intercept, and correlation coefficient are presented as mean ±standard deviation.

	Serum	Urine
LLOQ (ng/mL)	1.0 ng/mL	0.50 µg/mL
Linear range (ng/mL)	1.0-250 ng/mL	0.50-100 µg/mL
Slope (n = 3)	0.0026 ± 0.0001	0.0264 ± 0.0003
Intercept (n = 3)	0.0008 ± 0.0000	0.5638 ±0.0080
Correlation coefficient (R^2, n = 3)	0.9976 ± 0.0016	0.9992 ±0.0005

Table 3. Intra- and inter-day accuracy (%deviation) and precision (%CV) for LLOQ and QCs.

NMN	Level	Nominal Concentration	Intra-day[a]		Inter-day[b]	
			% Deviation	% CV	% Deviation	% CV
Serum (ng/mL)	LLOQ	1.0	1.3	10.7	0.9	9.8
	LQC	3.0	-4.1	3.8	-4.3	4.4
	MQC	75.0	-2.8	5.8	-3.3	5.8
	HQC	200	-6.7	5.4	-6.6	4.3
Urine (µg/mL)	LLOQ	0.5	1.7	3.1	-3.6	5.6
	LQC	1.5	-1.5	2.3	-4.2	4.8
	MQC	20.0	-4.9	1.9	-6.0	4.2
	HQC	80.0	-8.8	2.6	-8.3	3.1

[a] Three replicates for LLOQ; 12 replicates for QCs.
[b] Nine replicates for LLOQ; 24 replicates for QCs.

Table 4. Recovery and matrix effect of NMN from human serum and urine (n = 3).

NMN	QC Level	Nominal Concentration	Recovery (%, mean)	Matrix Effect (%, mean)
Serum (ng/mL)	LQC	3.0	100.3	99.7
	MQC	75.0	107.8	101.4
	HQC	200	98.1	97.0
Urine (µg/mL)	LQC	1.5	105.0	101.7
	MQC	20.0	97.7	92.2
	HQC	80.0	107.7	87.1

Table 5. Stability of NMN from human serum and urine (n = 3).

NMN	QC Level	Nominal Concentration	Bench Top Stability (RT, after 4 h)	Autosampler Stability (10°C, after 72 h)	Freeze/Thaw Stability (-80°C, after 3 cycles)
Serum (ng/mL)	LQC	3.0	92.1	99.7	99.0
	HQC	200	90.1	102.6	100.0
Urine (µg/mL)	LQC	1.5	104.0	105.3	103.5
	HQC	80.0	98.3	108.7	101.4

RESULTS

Figure 2. EICs for a) 1-methylnicotinamide and b) 1-methylnicotinamide-*d*3 for the low, middle, and high quality control samples as well as in a human serum sample.

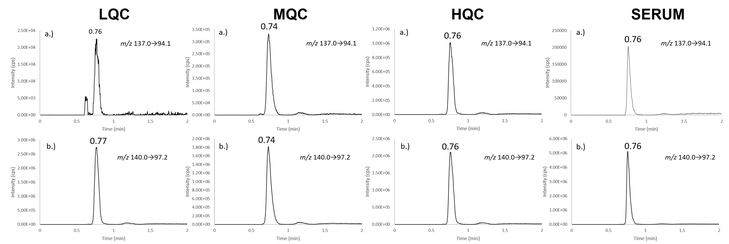
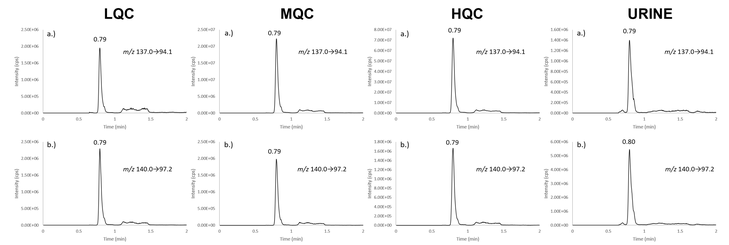


Figure 3. EICs for a) 1-methylnicotinamide and b) 1-methylnicotinamide-*d*3 for the low, middle, and high quality control samples as well as in a human urine sample.



CONCLUSIONS

- A sensitive, simple, and high throughput UPLC-MS/MS assay for the quantification of NMN in human serum and urine was developed and comprehensively validated according to FDA guidelines.
- The assay has several advantages over existing assays including small sample volume requirements, minimal sample preparation, high-throughput capacity, accuracy, and precision.
- The assay is also advantageous given that NMN can be used as an endogenous probe instead of using exogenously administered compounds.
- The method was applied to measure NMN in serum and urine for a clinical study with patients with chronic kidney disease.

ACKNOWLEDGMENT

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