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Scientific Report

Chemical Identification and Purity Determination of β-Nicotinamide Mononucleotide by NMR Spectroscopy

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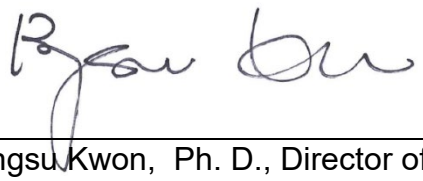
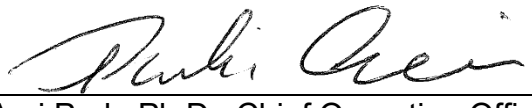
Natural Pet. Health

Author: 

09/18/2023

Byungsu Kwon, Ph. D., Director of NMR — Date

Reviewer: 

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Aeri Park, Ph.D., Chief Operating Officer — Date

1. Introduction

Natural Pet. Health submitted β-nicotinamide mononucleotide (NMN) (**Figure 1**), lot B220634F for chemical identification and purity determination by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The acquired ^{1}H NMR spectrum of the submitted sample was compared to its reference ^{1}H NMR spectrum for chemical identification (1). The purity determination of the sample was performed by quantitative NMR (qNMR) analysis using di-methyl sulfone as an internal standard. The client submitted two identical samples (TCL20600 and TCL20601), but only TCL20600 was utilized for NMR analysis. The detailed sample information is summarized in **Table 1**. Throughout this report, the β-nicotinamide mononucleotide, lot B220634F will be referred to as NMN.

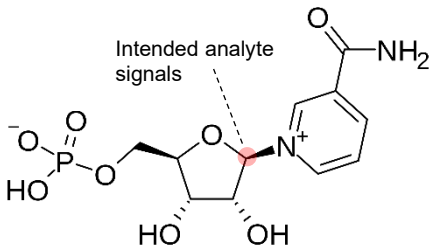


Figure 1. The chemical structure of β-nicotinamide mononucleotide.

Table 1. Summary of samples analyzed by ^{1}H NMR and qNMR spectroscopy

Triclinic Labs' Identifier	Compound	Lot Number	Sample ID	NMR Filename	Analysis
20600	β-nicotinamide mononucleotide	B220634F	1288-60-1	NMR1-3609	Chemical ID/Purity
20601	β-nicotinamide mononucleotide	B220634F	-	-	-

2. Results

The ^{1}H NMR spectrum (**Figure 2**) of NMN was compared with its reference ^{1}H NMR spectrum (**Figure 3**) (1). The chemical shifts (peak positions) and integrations of the acquired spectrum correspond to its reference spectrum, confirming the chemical identity. There are no visible impurities or residual solvents present in the NMR spectrum. **Table 2** summarizes the qNMR results showing the purity of NMN. A single measurement was performed[1] (**Figure 4**) using dimethyl sulfone[2] as an internal standard. The proton signal in the ribose sugar ring (**Figure 1**) at 6.25 ppm (doublet) were chosen due to its distinct separation from other peaks in the

[1] Typical qNMR analysis is performed by triplicate analysis, however the client requested a single measurement.
[2] The chemical shift of dimethyl sulfone (3.17 ppm) does not interfere with the analyte peaks.

spectrum. Based on the qNMR results, the purity of NMN is approximately 98.5%. The qNMR equation is below:

$$P_a(purity) = \frac{A_a}{A_{IS}} * \frac{N_{IS}}{N_a} * \frac{Wt_{IS}}{Wt_a} * \frac{MW_a}{MW_{IS}} * P_{IS}$$

Where, P_a is the purity of the analyte, A_a is integral of the analyte, A_{IS} is integral of the IS, N_{IS} is the number of IS protons, N_a is the number of analyte protons, Wt_{IS} is mass of the IS, Wt_a is mass of the analyte, MW_a is the molecular weight of the analyte, MW_{IS} is the molecular weight of the IS, and P_{IS} is the purity of the IS.

Table 2: Determination of purity of β-nicotinamide mononucleotide, lot B220634F.

Sample ID	Wt_a	A_a	A_{IS}	N_a	N_{IS}	MW_a	MW_{IS}	Wt_{IS}	$P_{IS\ (\%)}$	$P_{a\ (\%)}$
1288-60-1	34.994	5.38	6	1	6	334.22	94.13	1.804	100	98.5

3. Conclusion

The chemical identity of β-nicotinamide mononucleotide, lot B220634F was confirmed, and no visible impurities and residual solvents are present in the ^{1}H NMR spectrum. The purity was determined to be 98.5% by NMR spectroscopy analysis.

4. References

1. https://www.nutralion.com/product/%CE%B2-nicotinamide-mononucleotide-nmn/.

5. Experimental

^{1}H NMR Spectroscopy

The ^{1}H NMR spectrum was acquired on a Bruker NEO 400 MHz spectrometer using TopSpin GxP 4.1.4 software at Triclinic Labs. The acquired spectrum was processed using TopSpin GxP 4.1.4 and referenced to the chemical shift of the residual solvent peak (e.g., D_2O at 4.79 ppm). More detailed NMR sample preparation and acquisition parameters are provided in **Tables 3 and 4**. The NMR sample was prepared under ambient laboratory conditions.

Table 3: NMR sample preparation

TCL Number	Sample ID	Sample Preparation
TCL20600	1288-60-1	34.9935 mg of TCL20600 was dissolved in a mixture of 0.5 mL of dimethyl sulfone stock solution[3] and 0.5 mL of D_2O.

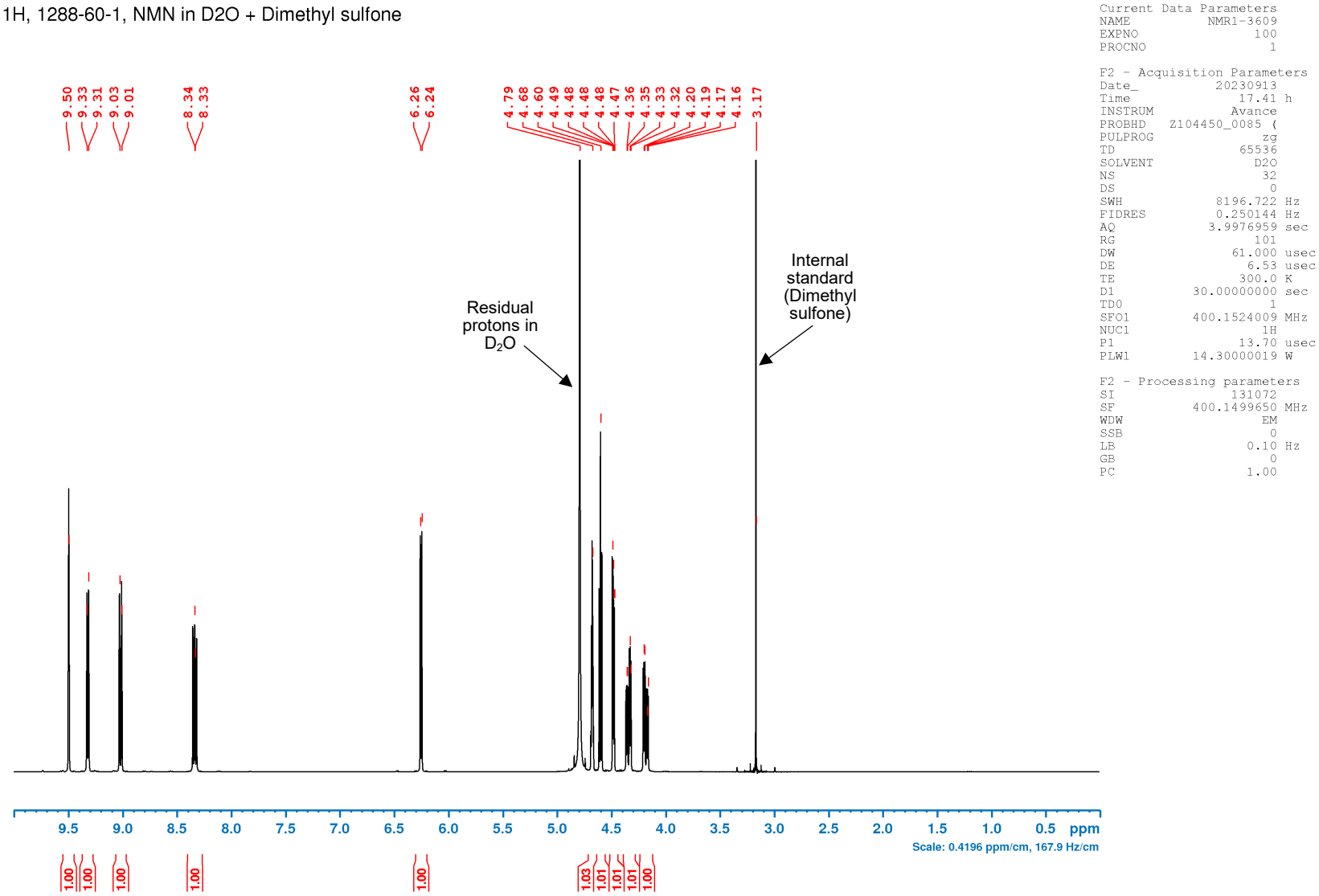
Table 4: Acquisition parameters

Parameter Name	Parameter Value
Sequence	zg
Size of FID (TD)	65536
Acquisition Time	4 sec
Spectral Width	8197 Hz
D1 (relaxation delay)	30 sec
Number of Scans	32
Transmitter Frequency	400.15 MHz
Transmitter Frequency Offset (O1P)	6.0 ppm
Line Broadening	0.1 Hz

[3] 18.04 mg of dimethyl sulfone was dissolved in 5 mL of D_2O.

6. Figures

Figure 2. The ^{1}H NMR spectrum of 1288-60-1 (β-nicotinamide mononucleotide, lot B220634F)

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EXPNO                      100
PROCNO                       1

F2 - Acquisition Parameters
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PULPROG                    zg
TD                      65536
SOLVENT                   D2O
NS                         32
DS                          0
SWH                  8196.722 Hz
FIDRES               0.250144 Hz
AQ                   3.9976959 sec
RG                        101
DW                     61.000 usec
DE                      6.53 usec
TE                     300.0 K
D1                30.00000000 sec
TD0                         1
SFO1               400.1524009 MHz
NUC1                       1H
P1                      13.70 usec
PLW1              14.30000019 W

F2 - Processing parameters
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SF                 400.1499650 MHz
WDW                        EM
SSB                         0
LB                       0.10 Hz
GB                          0
PC                       1.00
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Figure 3. The reference ^{1}H NMR spectrum of β-nicotinamide mononucleotide (1).

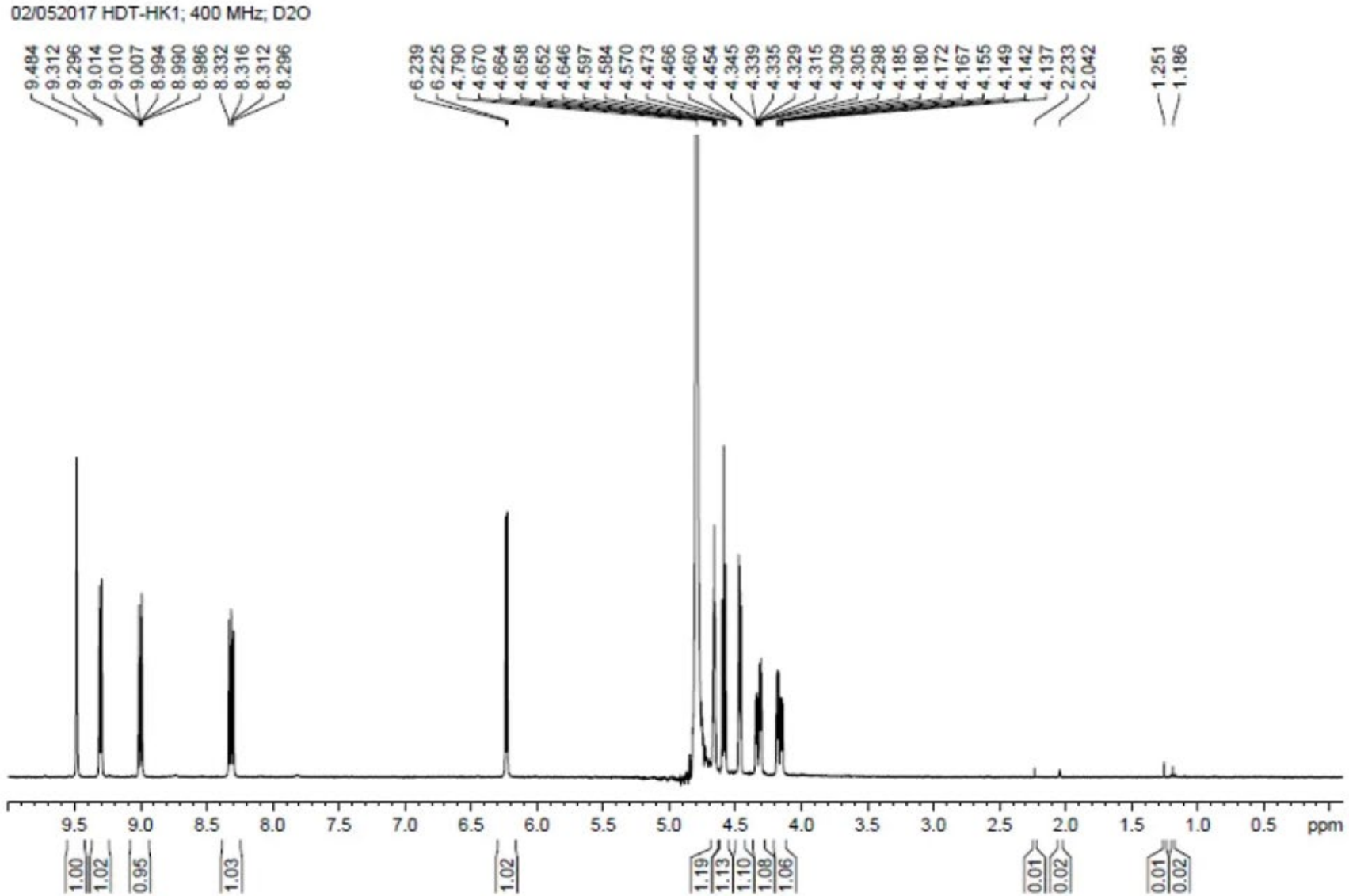

Figure 4. The ^{1}H qNMR spectrum of 1288-60-1 (β-nicotinamide mononucleotide, lot B220634F) with an internal standard. The integration values for the analyte and internal standard were utilized for purity determination.