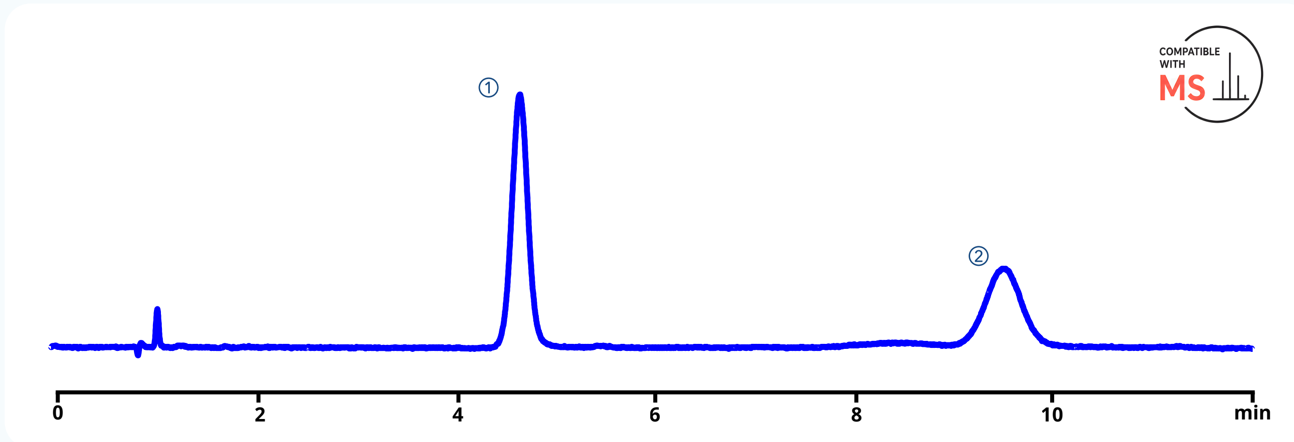
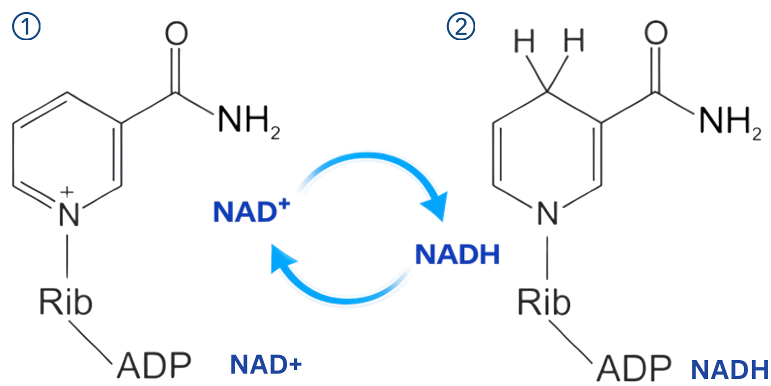
SIELC

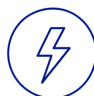
HPLC Method for Nicotinamide Adenine Dinucleotide (NAD), Nicotinamide Adenine Dinucleotide (reduced) (NADH) on PEI Column

JUNE, 2026





CONDITIONS	
Column	PEI
Column size	4.6 x 150 mm, 5 µm
Column part number	PEI-46.150.0510
Mobile phase	MeCN/H2O - 70/30%
Buffer	AmAc pH 6.8 - 60 mM
Flow rate	2.0 mL/min
Detection	260 nm


Cellular energy & ATP production

DNA repair & genomic stability

Methabolic pathways

Longevity & health aging

Application Comments

NAD and NADH are essential coenzymes involved in numerous biological and biochemical processes and have become important targets in pharmaceutical, biotechnology, metabolic, and aging-related research. Their analysis can be challenging because NAD and NADH readily undergo oxidation-reduction interconversion depending on sample preparation and handling conditions, potentially altering the NAD^+/NADH ratio and complicating accurate analysis.
The analysis was performed using the PEI stationary phase, which provides selective retention and separation of nucleotide compounds under MS-compatible mobile phase conditions. The selected conditions provide reproducible retention and adequate selectivity for NAD and NADH separation using UV detection at 260 nm. The presented chromatograms demonstrate stable retention, symmetrical peak shapes, and effective analyte separation, confirming the suitability of the method for reliable analysis and quantitative determination of NAD and NADH.

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