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SIELC

NICOTINAMIDE IN WHOLE BLOOD AND OTHER BIOLOGICAL SAMPLES

Nicotinamide, also known as niacinamide, is a form of vitamin B3 (niacin). In the body, nicotinamide plays a crucial role in energy production, DNA repair, and cell signaling. It is a precursor to nicotinamide adenine dinucleotide (NAD+), a coenzyme essential in cellular metabolism and other processes.

Nicotinamide can be found in various foods, including meat, fish, nuts, and green vegetables. Nicotinamide deficiency can lead to a condition known as pellagra, characterized by inflamed skin, diarrhea, and dementia. On the other hand, excessive intake can cause various adverse effects such as liver damage and gastrointestinal issues.

Accurate analysis of nicotinamide levels in different biological samples is essential for research and clinical diagnostics. SIELC developed an advanced method utilizing High-Performance Liquid Chromatography (HPLC) with our specialized columns to offer precise and reliable quantification of nicotinamide in diverse sample types such as blood, milk, and liver.

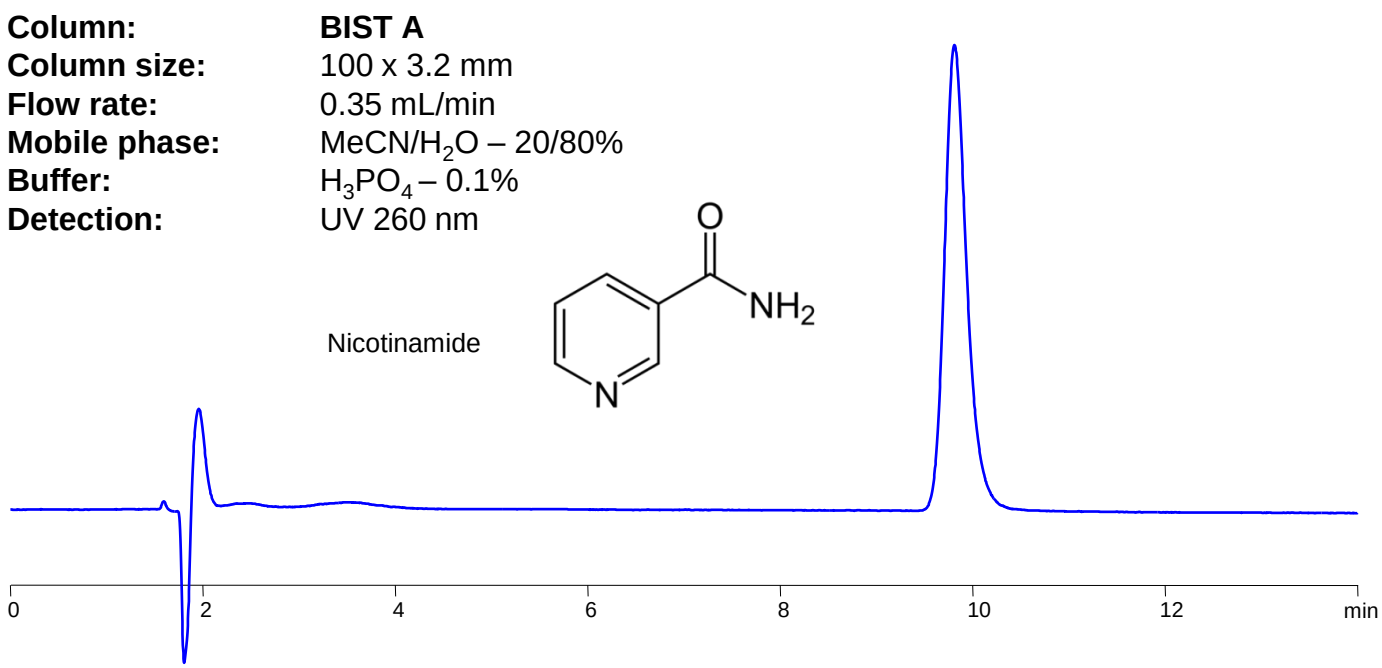


Figure 1. Retention of Nicotinamide on BIST A column

BIST A is a silica-based cation-exchange column with a negatively-charged surface and hydrophilic properties. Nicotinamide can be retained and analyzed on the BIST A column using a simple mobile phase consisting of acetonitrile (MeCN), water (H_2O), and phosphoric acid (H_3PO_4), as represented in Figure 1.

Nicotinamide is commonly found in complex matrices like blood, which contains proteins, lipids, and various other compounds that complicate analysis. Standard protein precipitation techniques often leave residual organic molecules that can interfere with the analysis and affect column performance. BIST A column provides an effective solution with its hydrophilic properties, designed to exclude hydrophobic compounds while providing a high degree of selectivity for hydrophilic compounds. This enhances the accuracy and reliability of nicotinamide detection. Figure 2 shows the chromatogram of a prepared blood sample with the isolated nicotinamide peak.

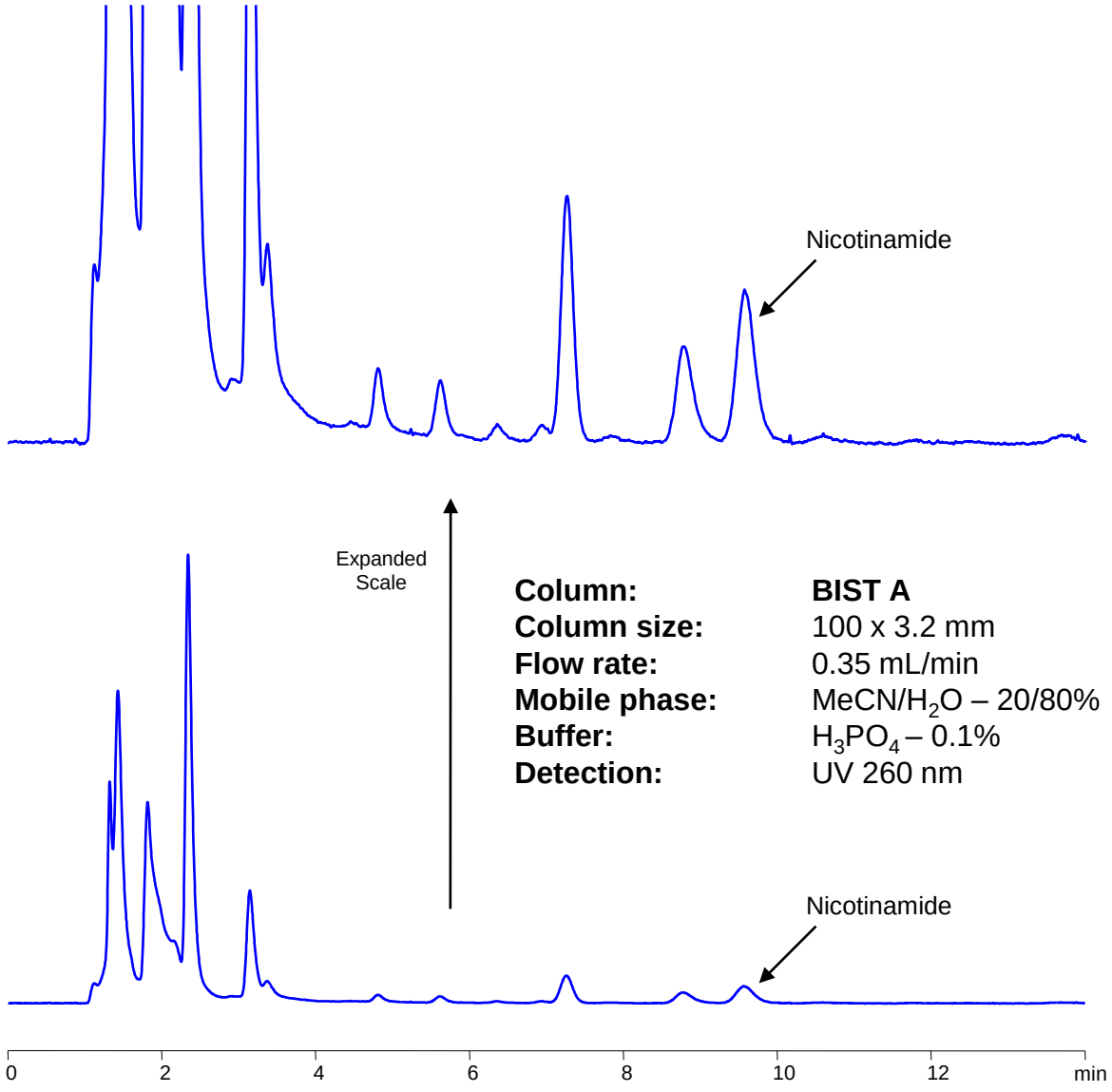


Figure 2. Chromatogram of a Prepared Blood Sample for Quantification of Nicotinamide

Sample Preparation and Nicotinamide Extraction from Whole Blood (by processing both cellular and liquid components of blood)

This extraction procedure involves extracting nicotinamide from both plasma (the liquid component of blood) and blood cells, such as red blood cells. The procedure includes lysing the cells to release their contents, including stored nicotinamide, and then removing the cellular debris.

In contrast, when plasma or blood serum extraction is performed, the cells are removed before any contents are released. Thus, the extracted compounds represent the amounts and concentrations found only in the liquid components of the blood.

Here, the extraction procedure is designed to obtain nicotinamide from both blood cells and the liquid component of blood.

Preparation of Stock Solutions

Acetonitrile and Water Stock Solution (MeCN/H_2O - 35/65%)
A mixture of 35 mL of MeCN and 65 mL of H_2O is prepared.

Nicotinamide Standard Stock Solution (1 mg/mL)
10 mg of Nicotinamide is accurately weighed and dissolved in 10 mL of the MeCN/H_2O - 35/65% solution.

Heparin Stock Solution (0.1 mg/mL from Heparin Sodium Salt, 171 units/mg)
1 mg of Heparin Sodium Salt (171 units/mg) is accurately weighed and dissolved in 10 mL of water. For blood sample preparation, 0.2 mL (equivalent to 3.42 units) is used.

Nicotinamide Standard Preparation

0.1 mg/mL Sample:
100 µL of Nicotinamide stock solution is pipetted into a 2 mL autosampler vial and diluted with 900 µL of MeCN/H_2O - 35/65% solution.

0.01 mg/mL Sample:
100 µL of the 0.1 mg/mL Nicotinamide sample is pipetted into a 2 mL autosampler vial and diluted with 900 µL of MeCN/H_2O - 35/65% solution.

0.005 mg/mL Sample:
500 µL of the 0.01 mg/mL Nicotinamide sample is pipetted into a 2 mL autosampler vial and diluted with 500 µL of MeCN/H_2O - 35/65% solution.

Blood Sample Preparation and Nicotinamide Extraction

1. 2 mL polypropylene centrifuge tubes are prepared by accurately weighing and recording the weight.
2. Approximately 0.2 mL of blood is collected from the finger into the 2 mL centrifuge tube, and the weight is recorded for accurate volume estimation.
3. The volume of blood collected is calculated using the weight difference and the average blood density (1.06 g/mL).
4. Immediately, 0.2 mL of heparin solution is added to the tube and mixed.
5. The sample is placed in a freezer overnight.
6. The sample is thawed (preferably on a laboratory shaker to facilitate mixing during thawing).
7. 1 mL of MeCN is added to the thawed sample, and the mixture is manually shaken thoroughly.
8. The sample is centrifuged at 13,000 RPM for 10 minutes.
9. The clear supernatant is pipetted into a clean autosampler vial.
10. The collected supernatant is diluted 1:1 with H_2O (e.g., 0.2, 0.3, or 0.4 mL of the supernatant is diluted with an equal volume of H_2O).
11. The sample is prepared for injection into the HPLC instrument (injection volume: 20 µL).

Figure 3 shows the chromatogram of the prepared blood sample. In addition to nicotinamide, compounds such as tyrosine, phenylalanine, and tryptophan were identified in the sample. The method can also be used for analyzing these compounds.

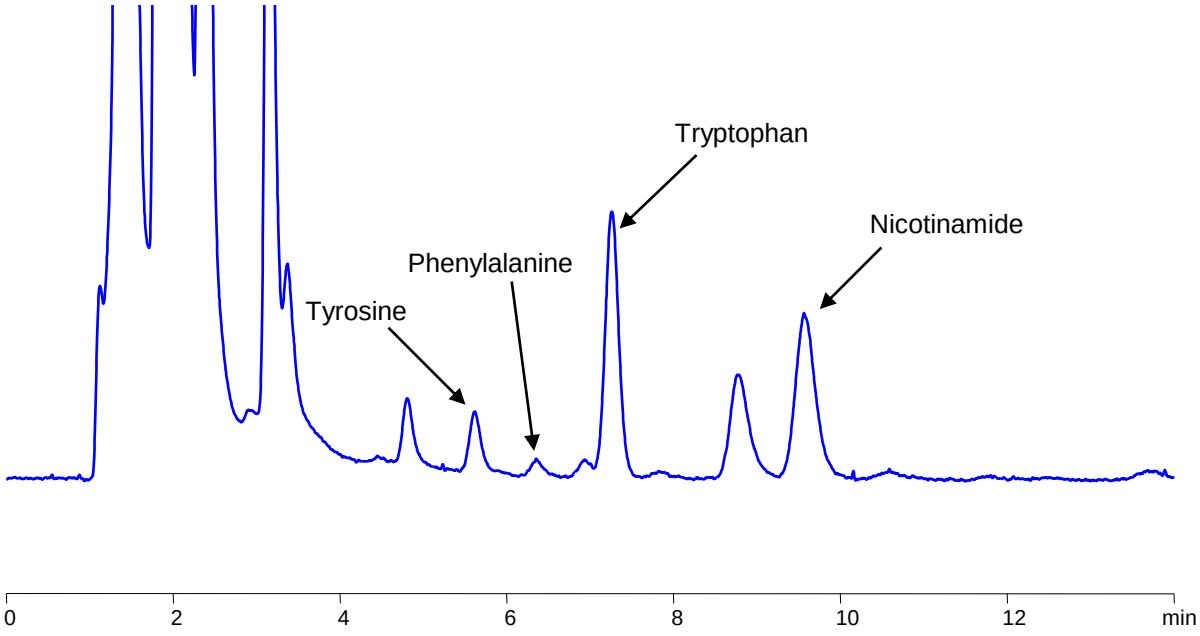


Figure 3. Chromatogram of a Prepared Blood Sample Identifying Tyrosine, Phenylalanine, and Tryptophan

Quantifying Nicotinamide Concentration in Blood Samples

•m_e **[g]** - weight of the empty centrifuge tube
•m_f **[g]** - weight of the centrifuge tube with collected blood (excluding added Heparin and MeCN)
•m_b **[g]** - weight of the collected blood, calculated as $m_f - m_e$
•d **[g/mL]** - average density of blood, 1.06 g/mL
•C_n **[mg/mL]** - concentration of the Nicotinamide standard
•A_n - area of the Nicotinamide standard
•A_d - area of Nicotinamide in the injected sample of diluted blood
•C_b **[mg/mL]** - concentration of Nicotinamide in the collected blood (calculated value of interest)
•V_b **[mL]** - volume of collected blood, calculated as m_b/d
•V_t **[mL]**- total volume of prepared blood (excluding dilution with water), calculated as V_i**+1.2 mL** (0.2 mL of Heparin solution and 1 mL of MeCN added)
•A_t - area of Nicotinamide in the total volume of prepared blood, calculated as $2 \times A_d$

Single Formula to Calculate the Concentration of Nicotinamide in Blood Sample

$$C_b = \frac{C_n \times A_t \times V_t}{V_b \times A_n}$$

Data and results below presents the analysis of nicotinamide levels across multiple blood samples obtained simultaneously from the same subject. The primary objective is to estimate the variability in nicotinamide concentration, based on the assumption that levels should be consistent across all samples, given that they were collected at the same time of day. Tables 1 and 2 present the initial nicotinamide standard and blood sample data, along with the initial calculations.

Table 1. Nicotinamide Standard

Standard	Concentration, **Cn [mg/mL]**	Area of Standard, **An**
Nicotinamide	***Cn [mg/mL]***	***An***
Nicotinamide	0.005	***631.56***

Table 2. Sample Preparation and Initial Calculations

Sample	Date Obtained	Freeze	Vial Empty Weight, **me [g]**	Vial Full Weight, **mf [g]**	Weight of Blood, **mb [g]**	Blood Average Density, **d [g/mL]**	Blood Volume, **Vb [mL]**	Heparin Standard Volume, **[mL]**	MeCN Volume, **[mL]**	Total Volume, **Vt [mL]**
A	MM/DD	Yes	***me***	***mf***	***mf - me***	1.06	***mb/d***	0.2	1	***Vb+1.2 mL***
A1	07/31	Yes	***0.9668***	***1.2131***	***0.2463***	1.06	***0.2324***	0.2	1	***1.4324***
A2	07/31	Yes	***0.9578***	***1.206***	***0.2482***	1.06	***0.2342***	0.2	1	***1.4342***
A3	07/31	Yes	***0.9947***	***1.2256***	***0.2309***	1.06	***0.2178***	0.2	1	***1.4178***
A4	07/31	Yes	***0.9948***	***1.3825***	***0.3877***	1.06	***0.3658***	0.2	1	***1.5658***

Table 3. Results		
Area of Diluted Sample, **Ad**	Area in Total Volume, **At**	Nicotinamide Concentration in Blood, **Cb [mg/mL]**
Ad	**2×Ad**	***Cb***
39.91	79.82	***0.003895***
39.74	79.48	***0.003854***
37.21	74.42	***0.003835***
56.82	113.64	***0.003851***

Table 4. Variability		
Average	STD.Dev	error, %
0.003859	***0.000026***	***0.668226***

Table 3 presents the final nicotinamide concentrations in blood samples, and Table 4 shows the variability across these samples. The data includes individual levels, as well as the average, standard deviation, and percentage error. The standard deviation indicates the spread around the average, while the % error reflects the relative magnitude of this variability. Low values for both metrics suggest minimal variability, supporting the consistency of nicotinamide levels in samples collected simultaneously.

Linearity Study of Nicotinamide Analysis Quantification Method

Figure 4 shows the constructed calibration curve and demonstrates the linearity of the nicotinamide response relative to its concentration. The Limit of Quantification (LOQ) achieved for nicotinamide is 0.02 ppm, indicating the lowest concentration at which the compound can be quantitatively detected with acceptable precision and accuracy.

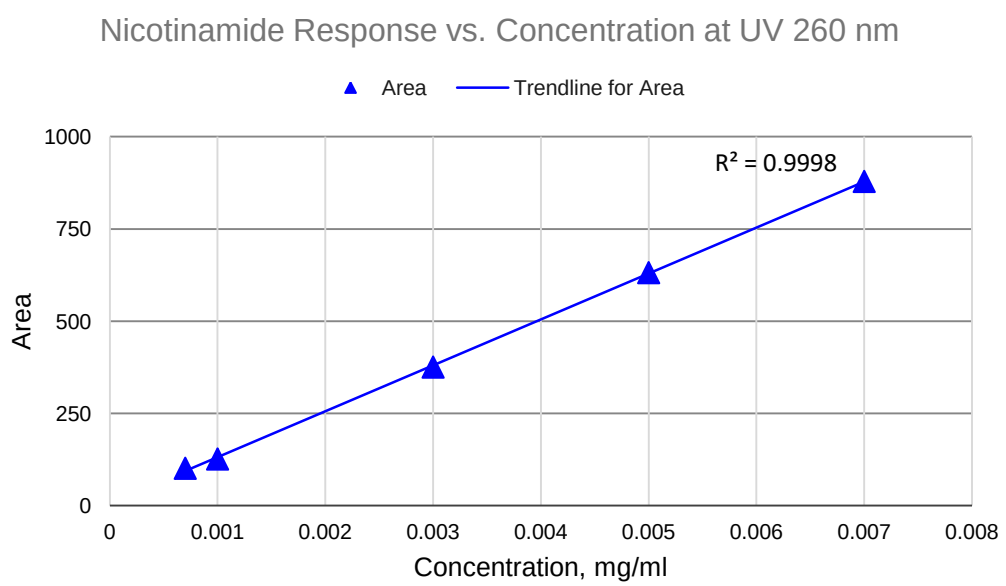
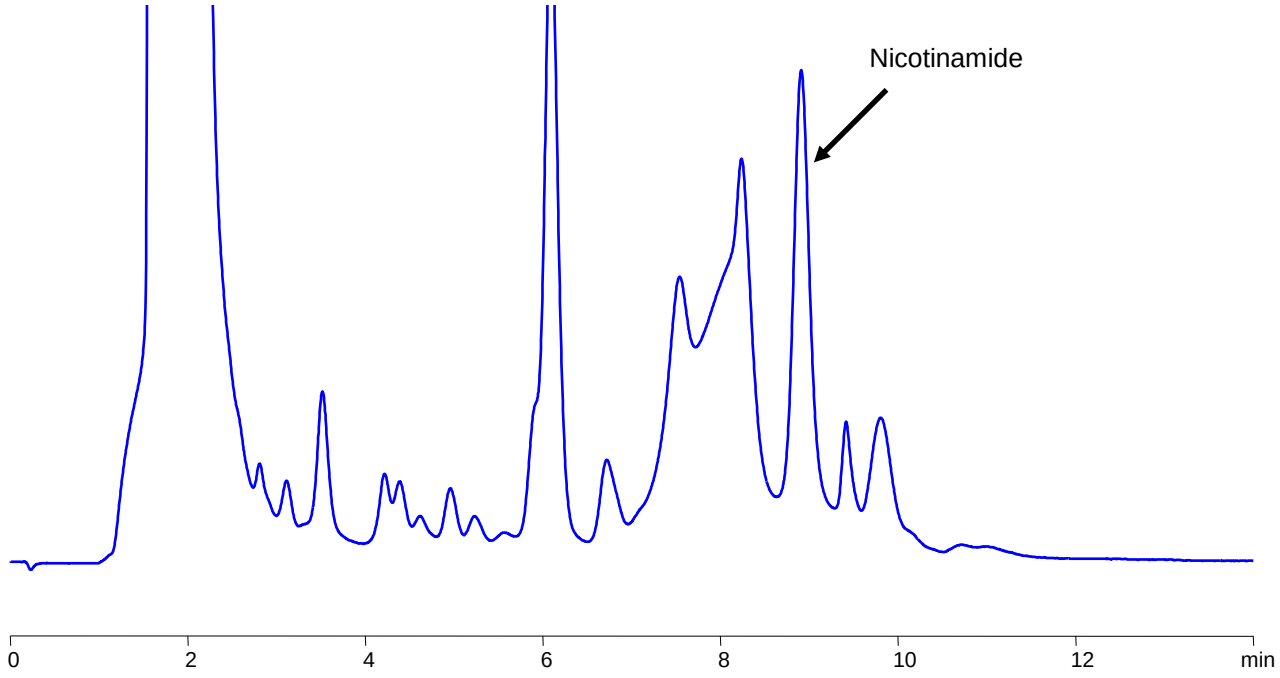
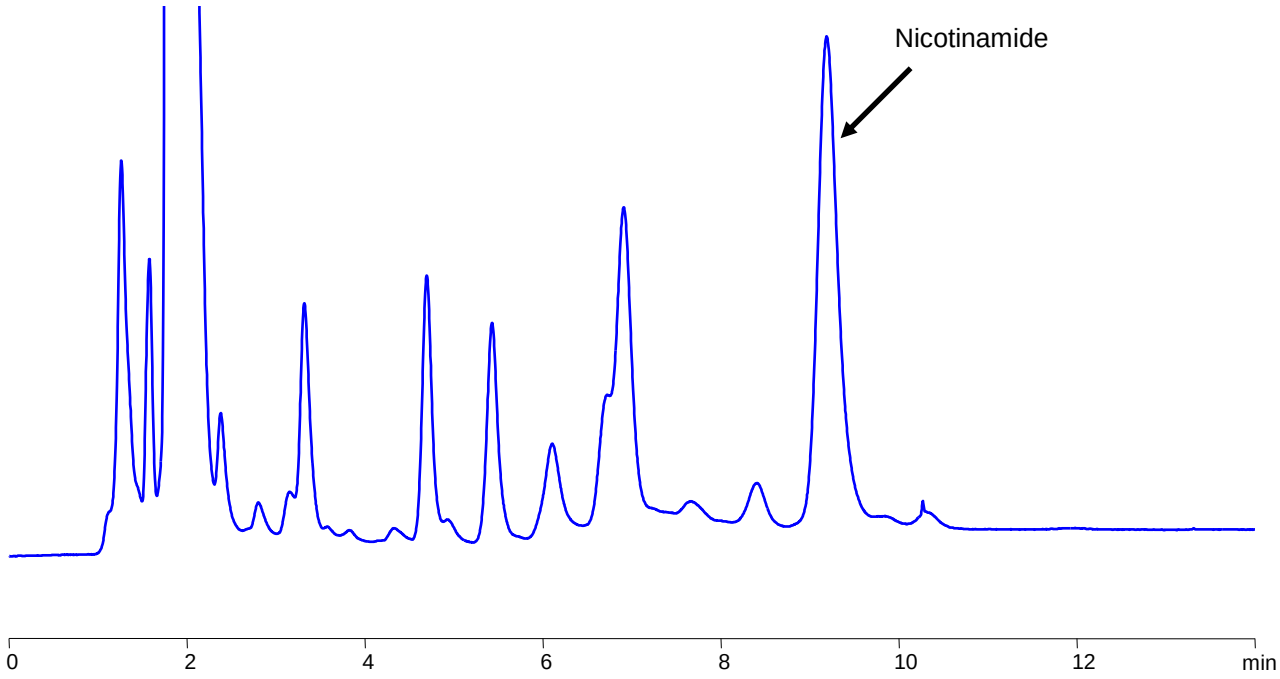


Figure 4. Linearity Study of Nicotinamide Analysis Quantification Method

Nicotinamide Content in Cow Milk



Nicotinamide Content in Cow Liver



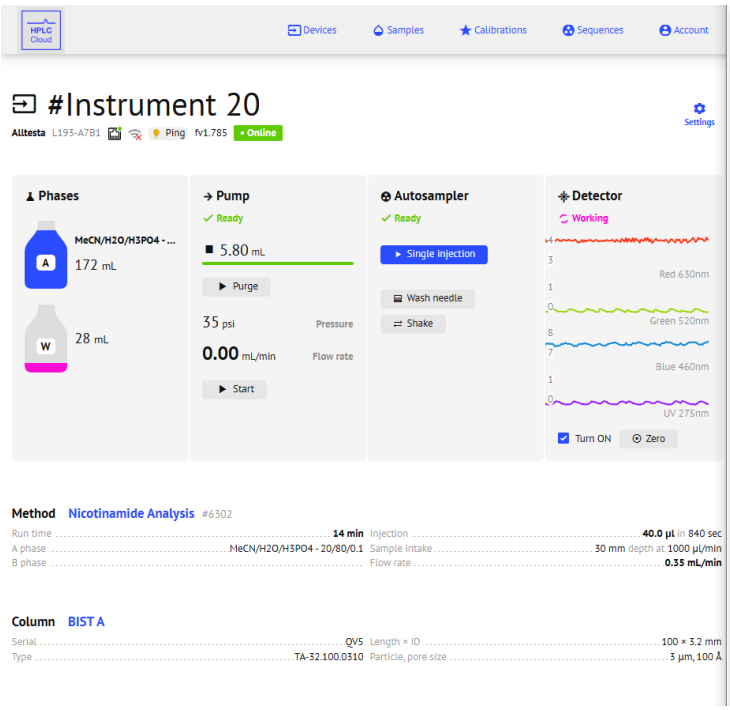
SIELC offers a range of affordable HPLC analytical system solutions. These mini-HPLC systems provide a convenient, tabletop solution for measuring various chemicals in small and trace amounts across different products. These systems transform complex analyses into simple, user-friendly processes. Compact yet fully equipped, the mini-HPLC instruments come with intuitive software, ensuring an excellent user experience.

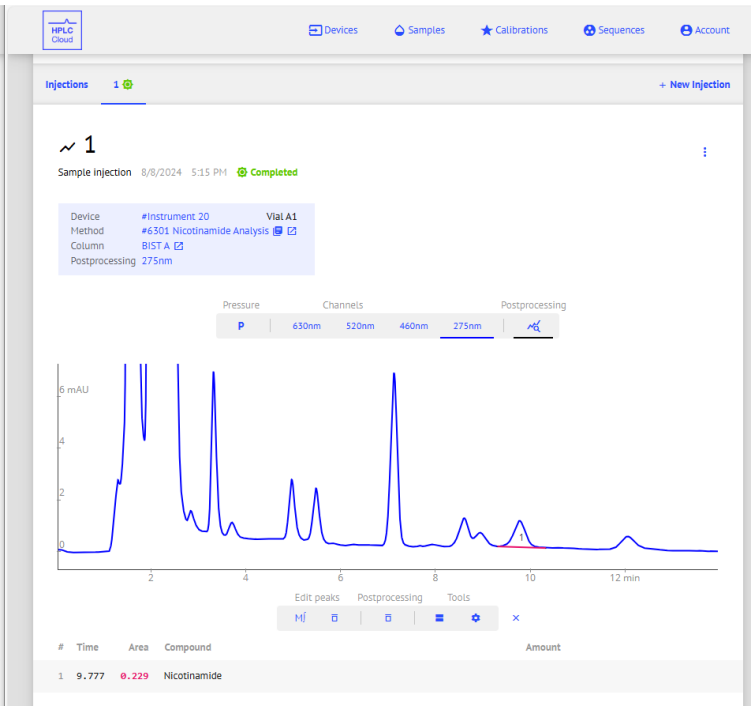
These mini-HPLC systems are also ideal for the precise and reliable quantification of nicotinamide in blood. Their advanced capabilities make them perfect for both research and clinical diagnostics, enabling accurate analysis of nicotinamide levels to support various health and scientific applications.

Click here to learn more about Alltesta™

Click here to learn more about Cromite™

SIELC has developed the first and only cloud-based HPLC software, streamlining analysis by connecting instruments directly to our secure server via Ethernet. All parameters are visible and adjustable on the fly from any internet-enabled device, making the process more efficient, user-friendly, and accessible from anywhere.





Analyzing Nicotinamide in Whole Blood using Alltesta™ and HPLC.cloud