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Sensitive Determination of Nisin in Food Products by Large Volume Sample Stacking-Capillary Electrophoresis

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Table of Contents

Abstract **2**

1. Introduction **3**

2. Materials and Methods **5**

2.1 Chemicals 5

2.2 Standard Solutions and Background Electrolytes 5

2.3 *Instrumentation and LVSS Method* 6

3. Results and Discussion **7**

3.1 LVSS Method Optimization 7

3.1.1 Method A (1-minute sample injection, -10 kV switch polarity) 7

3.1.2 Method B (2-minute sample injection, -10 kV switch polarity) 12

3.1.3 Method C (2-minute sample injection, -15 kV switch polarity) 16

3.1.4 Summary 20

3.2 Future Work 21

Conclusion **22**

Acknowledgements **22**

References: **23**

Abstract

Lantibiotics are a class of polycyclic peptide antibiotics with nisin being the sole FDA-approved. Lantibiotic nisin is used as a preservative in drinks, dairy, and meats. However, nisin stability is influenced by temperature, pH, and food components, demanding careful assessment. Current methods of nisin quantifications include immunoassays and bioassay which are limited by interference and sensitivity. Capillary electrophoresis (CE) is proposed as a novel method for trace nisin detection in dairy. CE offers high efficiency, speed, and minimal sample consumption. In this project, large-volume sample stacking (LVSS) was employed to increase the sensitivity of the method. Three methods were developed to determine the optimized parameters for nisin detection. Optimal results were achieved using a 2-min injection time and -15 kV polarity switch. This method incorporated the use of a background electrolyte consisting of 30 mM sodium phosphate dibasic at pH 3.75 with sample solvent composition of 10 mM sodium phosphate dibasic at pH 3.0. The optimized method shows promise for achieving the sensitivity requirements of the low-level nisin detection in food products.

1. Introduction

Lantibiotics are produced by Gram-positive bacteria. Lantibiotics can combat other bacteria through Lipid II in two ways; (1) to inhibit the synthesis of the peptidoglycan bacterial cell wall, or (2) as a docking molecule to facilitate pore formation triggering apoptosis (Delves-Broughton, 1996; Karpíski & Szkaradkiewicz, 2015). Nisin, a 1A lantibiotic from Lactococcus lactis, acts via both mechanisms (Silva et al., 2018), finding broad use as a preservative in alcoholic drinks, dairy, and meats (Delves-Broughton, 1996). Nisin is the only lantibiotic that is FDA approved and utilized as a biopreservative due to its low toxicity (Silva et al., 2018).

While effective, nisin's stability is affected by temperature, pH, and food components (Silva et al., 2018). To ensure effective usage, its quantity and stability need careful assessment (Hakovirta et al., 2006; Soliman & Donkor, 2010). Analytically, nisin quantification involves immunoassays (Leung et al., 2002) and bioassays (Hakovirta et al., 2006). Immunoassays, like ELISA, detect bioactive nisin and its degradation products (Leung et al., 2002), yet face interference from niacin-similar-compounds in foods. Bioassays are not preferred due to their lengthy and tedious workflow in addition to sensitivity and specificity issues (Fowler et al., 1975). Recognizing these constraints, this study proposes capillary electrophoresis (CE) as an alternative analytical approach for trace nisin detection in dairy products. Crafting a sensitive CE detection method would effectively bridge this gap, presenting a viable solution for the food industry's needs.

CE is a robust analytical technique, characterized by its exceptional separation efficiency, rapid analysis speed, minimal sample consumption, and potential for automation (Chen, Xu, Lin, & Chen, 2008; Quirino & Terabe, 2000). CE's advantage lies in its minute sample injection compared to techniques like HPLC, although this small injection volume sacrifices sensitivity. Additionally, the short optical path further hampers CE's sensitivity when detecting analytes at ultralow concentrations, such as nisin in dairy matrices.

To enhance CE's sensitivity, the integration of the large-volume sample stacking (LVSS) with switch polarity preconcentration technique has been employed. Traditional CE involves injecting a small sample plug (e.g., 0.5-1.0 psi for 5-10s or 1-2% of capillary length). Conversely, LVSS introduces a large sample plug (e.g., 15 psi for 60s or up to 80% of capillary length). The large sample volume increases sensitivity however sacrifices resolution. To address the resolution, LVSS utilises a polarity switch directly following sample injection and induces a reduced electric field. The polarity switch and induced field leads to analyte concentration within a confined zone. This process augments the detection signal, ultimately enhancing method sensitivity (Šlampová, Malá, & Gebauer, 2019). The LVSS technique is summarized in *Figure 1*.

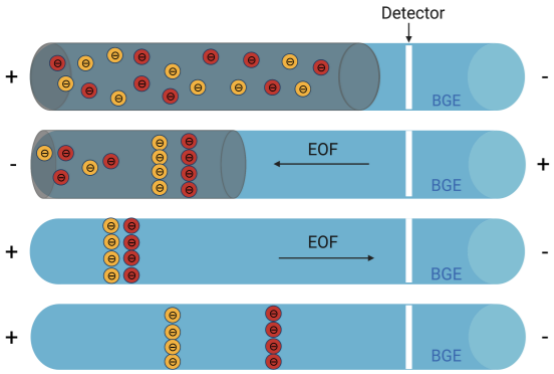

Figure 1: Schematic of Large Volume Sample Stacking (LVSS) adapted from Islas et al. 2018.

The aim of this study was to improve a sensitive LVSS method for the detection of nisin at the parts-per-billions (ppb) level in food products. To achieve this goal, two experimental parameters were optimized. The two experimental parameters optimized were, sample injection time and switch polarity voltage.

To achieve this goal, three methods were tested. The first method, referred to as method A, was composed of a 1-minute sample injection time and -10 kV switch polarity. The second method, referred to as method B, was composed of a 2-minute sample injection time and -10

kV switch polarity. Increasing the sample injection time is expected to increase the analyte signal due to the presence of more analyte. The final method, referred to as method C, was composed of a 2-minute sample injection time as well as -15 kV switch polarity. The higher the applied voltage, the faster the analysis and the higher the efficiencies and resolution. This is only true up to a certain point. As the voltage is increased, Joule heating is generated in the capillary resulting in negative components such as broader peaks, sample decomposition, or formations of bubbles inside the capillary.

2. Materials and Methods

2.1 Chemicals

Nisin from *Lactococcus Lacti*s was purchased from Sigma-Aldrich Chemical Company and stored at 4 °C according to the manufacture's instructions. Sodium phosphate dibasic was purchased from Caledon Laboratories (Georgetown, ON, CA) and was used to prepare the diluent for the nisin standard solutions as well as the background electrolyte (BGE). Additionally, 18 MΩ water was used to prepare the diluent solution as well as the BGE.

2.2 Standard Solutions and Background Electrolytes

To prepare the diluent solution, an appropriate amount of sodium phosphate dibasic was weighed and dissolved using 18 MΩ water to achieve a final concentration of 10 mM. Then, 0.1 M HCl was used to adjust the pH to 3.0 (±0.1). The pH was measured using a SevenCompound™ Duo S213. The solution was then filtered using 0.45 µm membrane filters and transferred to a clean, dry, labelled plastic bottle. The plastic bottle was kept at room temperature.

To prepare the BGE, an appropriate amount of sodium phosphate dibasic was weighed and dissolved using 18 MΩ water to achieve a final concentration of 30 mM. Then, 0.1 M HCl was used to adjust the pH to 3.75 (±0.1). The pH was measured using a SevenCompound™ Duo

S213. The solution was then filtered using 0.45 µm Nylon membrane filters and transferred to a clean, dry, labelled plastic bottle. The plastic bottle was kept at room temperature.

To prepare the nisin 1000 mg L^{-1} stock solution, an appropriate amount of nisin was weighed out and made to volume using the diluent solution. The solution was vortexed to ensure complete dissolution of the nisin. The solution was then filtered using 0.45 µm membrane filters and transferred to a clean, dry, labelled glass bottle and kept at 4^{o}C to preserve the sample. Nisin standard solutions were prepared by dissolving appropriate amounts of the nisin stock solution and diluting them using the diluent solution to achieve concentrations of 150 mg L^{-1}, 100 mg L^{-1}, 50 mg L^{-1}, and 25 mg L^{-1}.

2.3 *Instrumentation and LVSS Method*

The nisin stock solutions were analyzed using a SCIEX P/ACE MDQ Plus capillary electrophoresis system equipped with a UV detector. Data was acquired using 32Karat software. The detector wavelength used for all runs was 214 nm. Samples were injected to the capillary using 15 psi for all runs. All runs were separated using 10 kV polarity for 3 min and switch polarity for 30 min. The separations were carried out in a fused-silica capillary with an internal diameter of 50 μm. The fused-silica capillary was housed in a cartridge and kept at a temperature of 25 °C by a liquid fluorocarbon coolant system. The sample injection time and switch polarity voltages varied throughout the three methods studied. Capillaries were rinsed prior to each experimental run. This pre-run rinse consisted of 0.1 M NaOH, 18 MΩ water, and BGE all at 20 psi for 3 min each. The instrument parameters as well as the differences between the methods can be seen in *Table 1*.

Method	A	B	C
UV Detector Wavelength	214 nm	214 nm	214 nm
Capillary Diameter	50 µm	50 µm	50 µm
Effective Capillary Length	50 cm	50 cm	50 cm
Experimental Temperature	25°C	25°C	25°C
Sample Injection Time	1 min	1 min	2 min
Sample Injection Pressure	15 psi	16 psi	17 psi
Applied Voltage (Normal)	10 kV	10 kV	10 kV
Applied Voltage (Reverse)	-10 kV	-15 kV	-15 kV
Switch Polarity Time	30 min	30 min	30 min

Table 1: Instrument Parameters for all three methods studied.

3. Results and Discussion

3.1 LVSS Method Optimization

In this study, three optimization methods were tested and compared for the detection of nisin. Nisin standards of known concentrations (150 mg L^{-1}, 100 mg L^{-1}, 50 mg L^{-1}, and 25 mg L^{-1}) were produced on the same day as the experimental runs used to test the methods. The same set of standards were used on each method and were prepared in 10 mM sodium phosphate dibasic at pH 3.0. The BGE used for all runs was 30 mM sodium phosphate dibasic at pH 3.75. All three methods consisted of a pre rinse of capillary prior to experimental run, sample injection with 15 psi, 10 kV normal polarity for 3 min and switch polarity for 30 min. The differences between the three methods lies in the sample injection time and the switch polarity voltage.

3.1.1 Method A (1-minute sample injection, -10 kV switch polarity)

For this method, the sample injection time is 1 min, and the switch polarity voltage used is -10 kV. This method yielded good peaks indicating good resolution. The presence of nisin is confirmed as the signal (peak height) increases with increasing nisin concentration and decreases with a decrease in nisin concentration *Figures 2-5*. Additionally, the migration time of

nisin in all the experimental runs is quite consistent. Furthermore, the method shows some linearity as shown in *Figure 6*. Linearity represents that the results are directly proportional to concentration. However, this method does not yield an instrument signal that is fit for the purpose of detecting nisin at the ppb level.

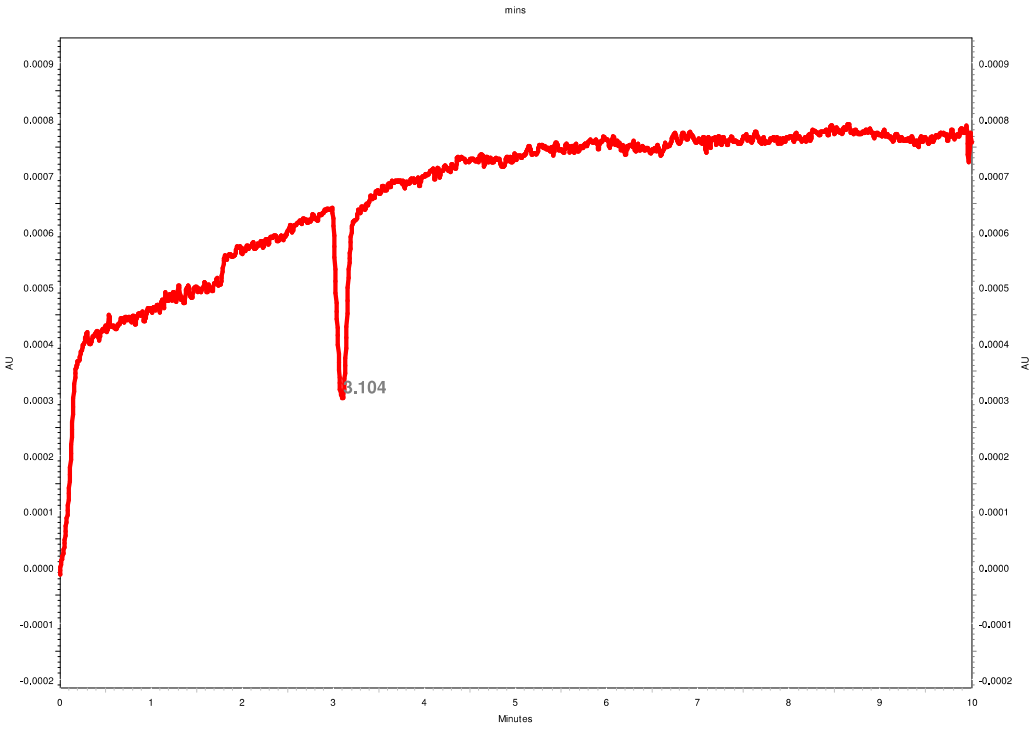

Figure 2: Electropherogram of 25 mg/L nisin standard using method A (peak height 339 AU)

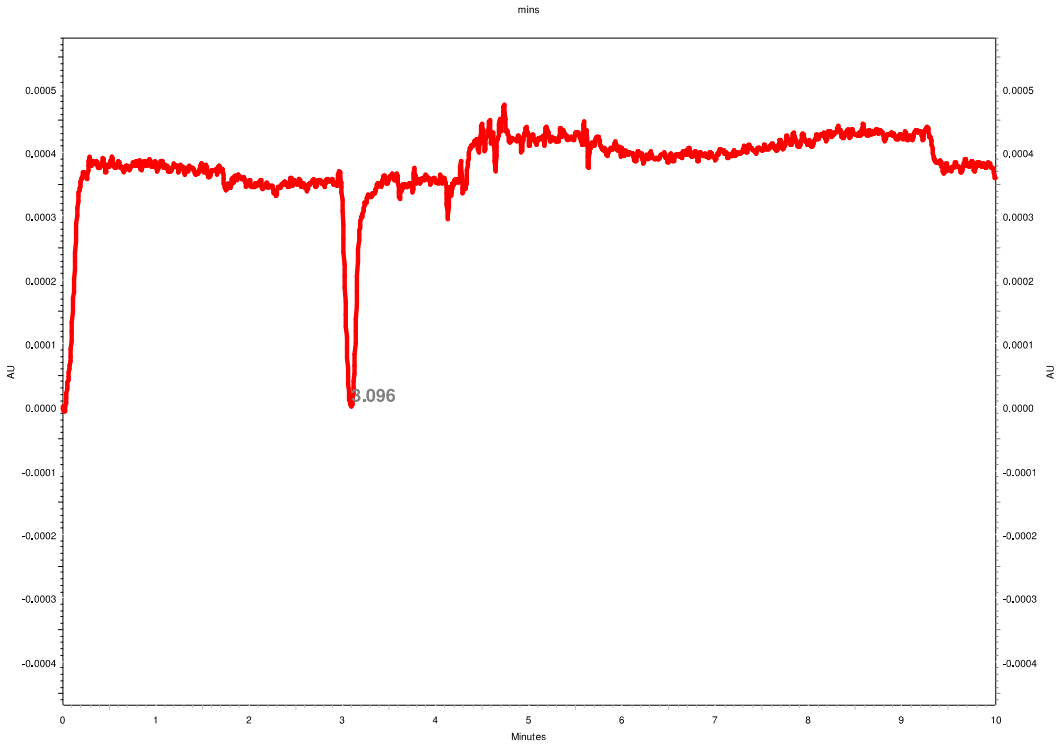

Figure 3: Electropherogram of 50 mg/L nisin standard using method A (peak height 350 AU)

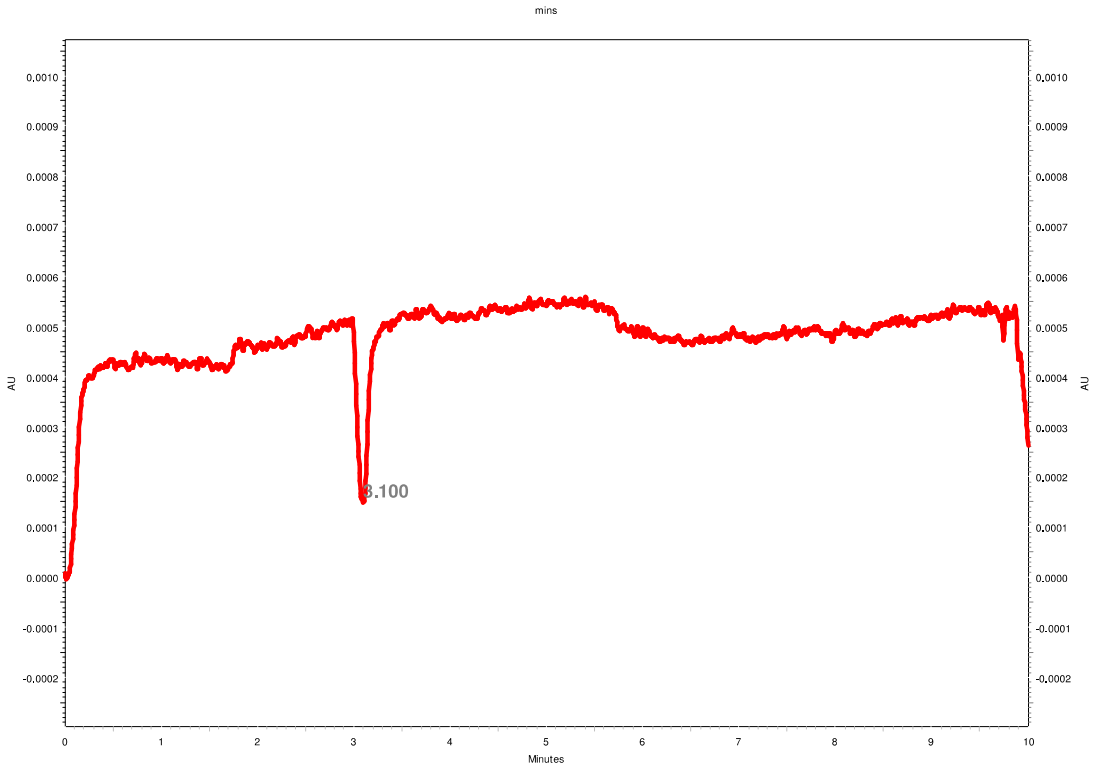

Figure 4: Electropherogram of 100 mg/L nisin standard using method A (peak height 353 AU)

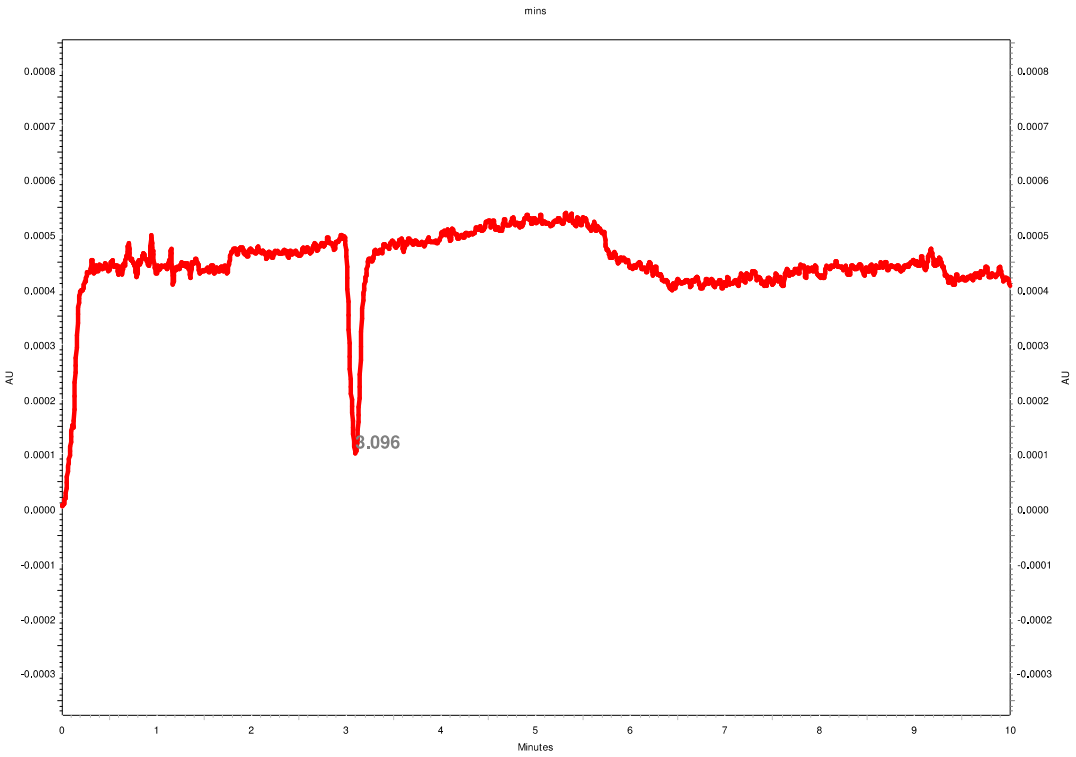

Figure 5: Electropherogram of 150 mg/L nisin standard using method A (peak height 376 AU)

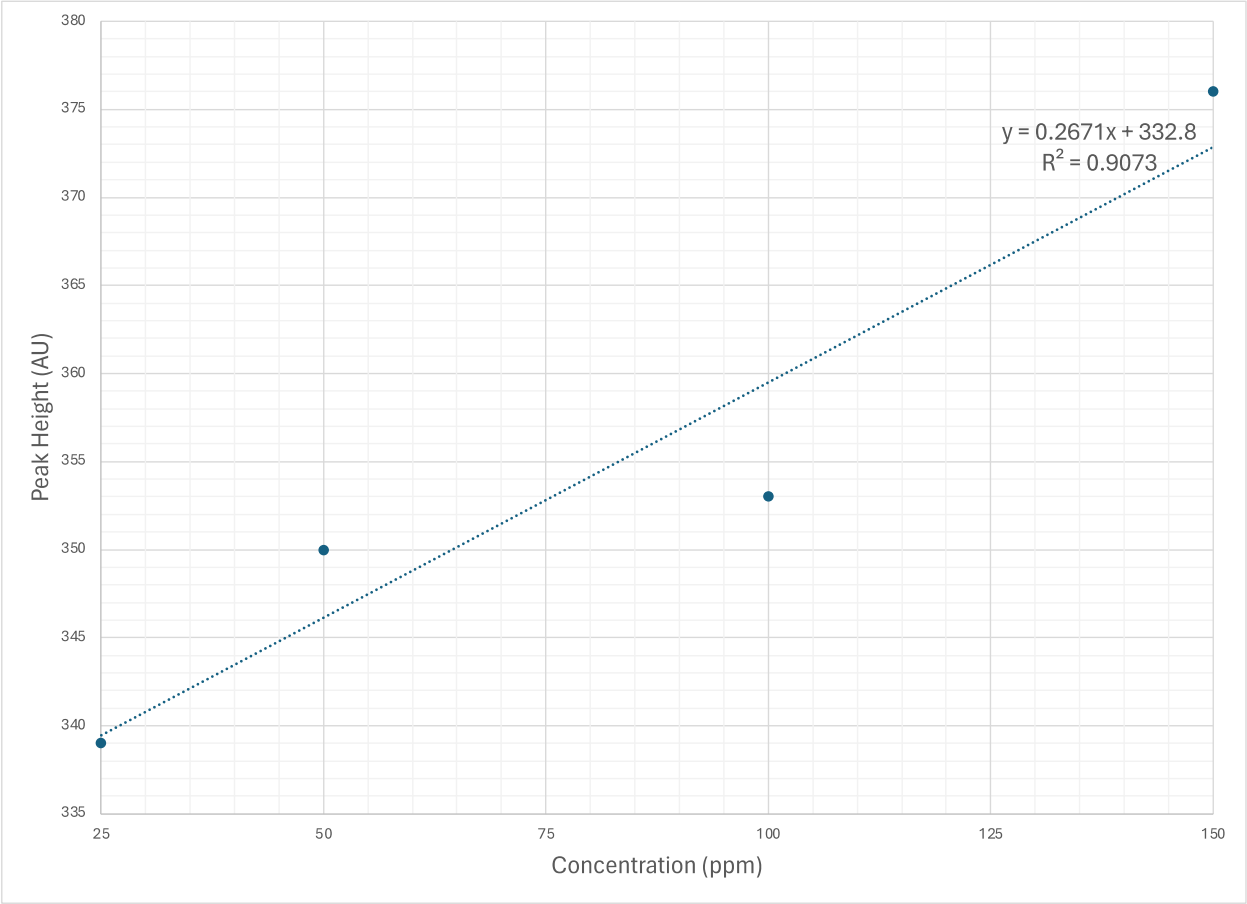

Figure 6: Calibration curve of Nisin Standards of concentrations (150 mg L^{-1}, 100 mg L^{-1}, 50 mg L^{-1}, and 25 mg L^{-1}) using Method A.

3.1.2 Method B (2-minute sample injection, -10 kV switch polarity)

For method B, the sample was injected for 2 min, as opposed to 1 min from the previous method. The switch polarity used was -10 kV. An increased sample injection time is expected to yield an increase in signal as there is more analyte present. However, this was not the case. In *Table 1,* we can see that the values of the peak heights observed in this method are all lower than the peak heights observed from method A. This method did not yield significant results that could be used to achieve the goal of this study in detecting nisin at the ppb level.

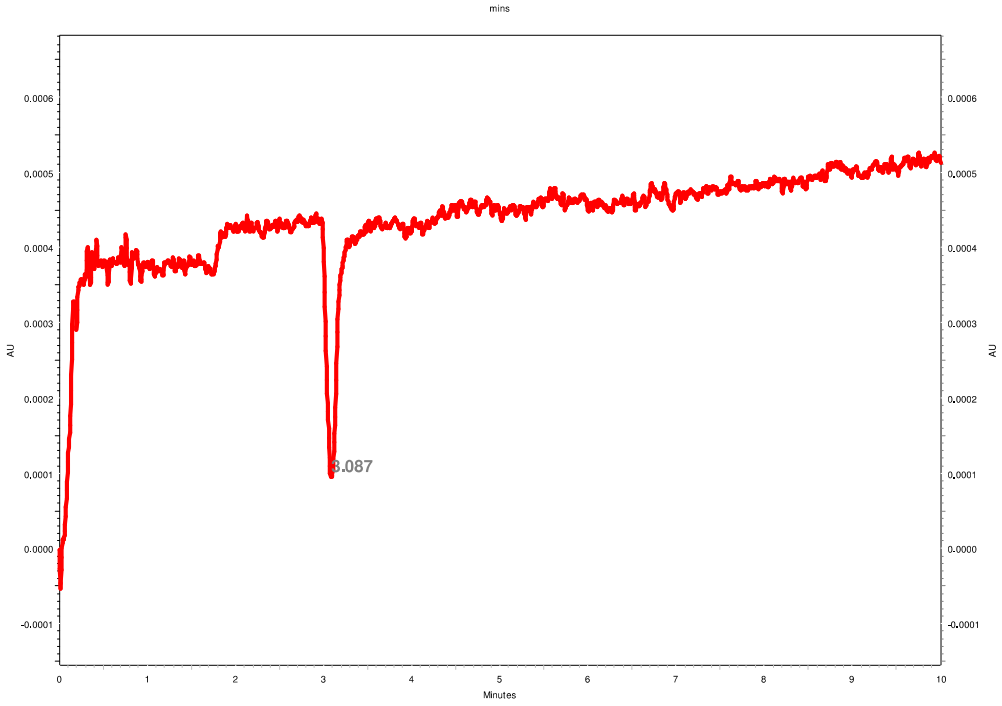

Figure 7: Electropherogram of 25 mg/L nisin standard using method B (peak height 333AU)

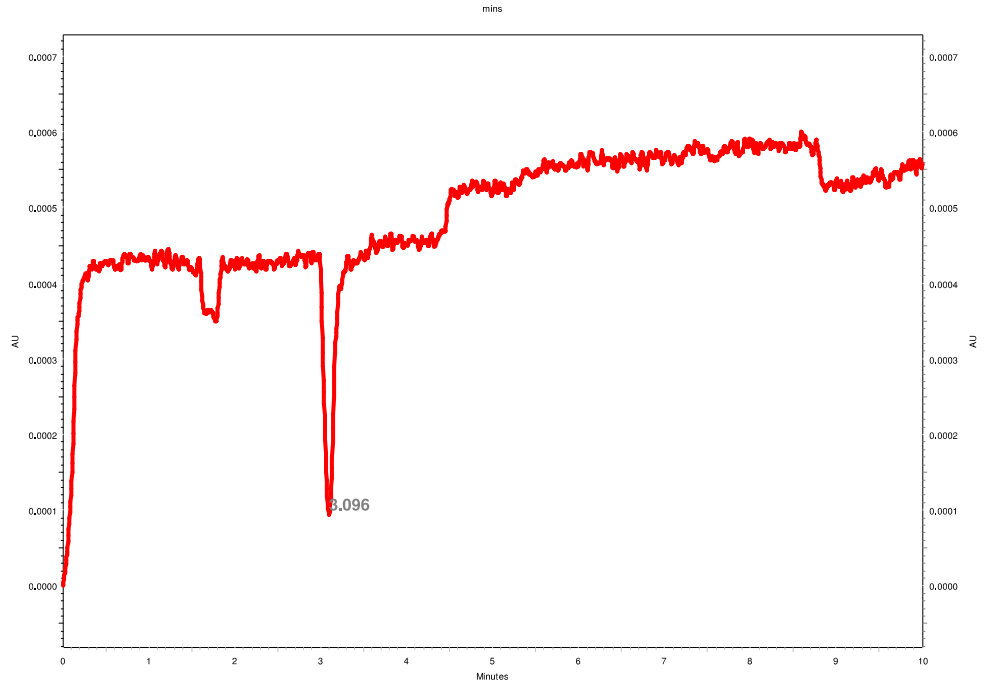

Figure 8: Electropherogram of 50 mg/L nisin standard using method B (peak height 339 AU)

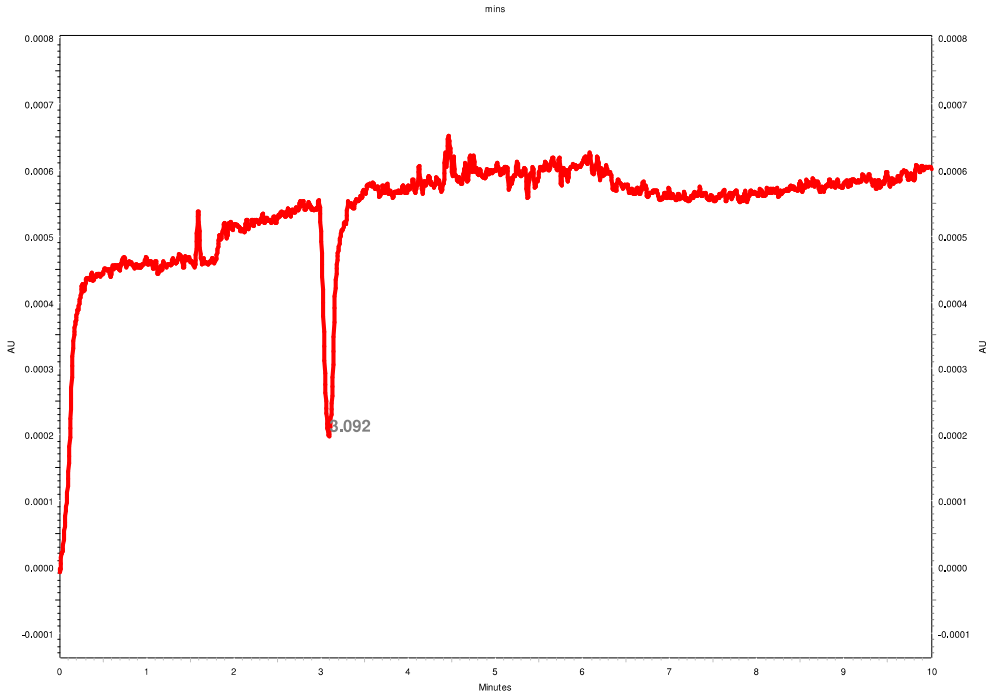

Figure 9: Electropherogram of 100 mg/L nisin standard using method B (peak height 346 AU)

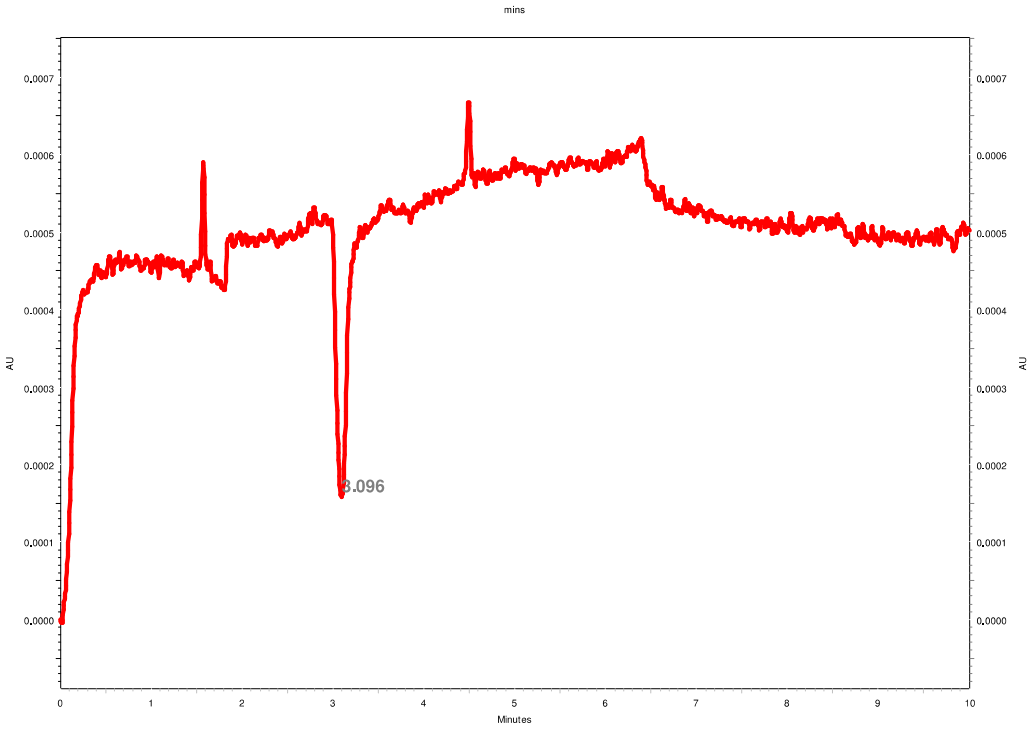

Figure 10: Electropherogram of 150 mg/L nisin standard using method B (peak height 351 AU)

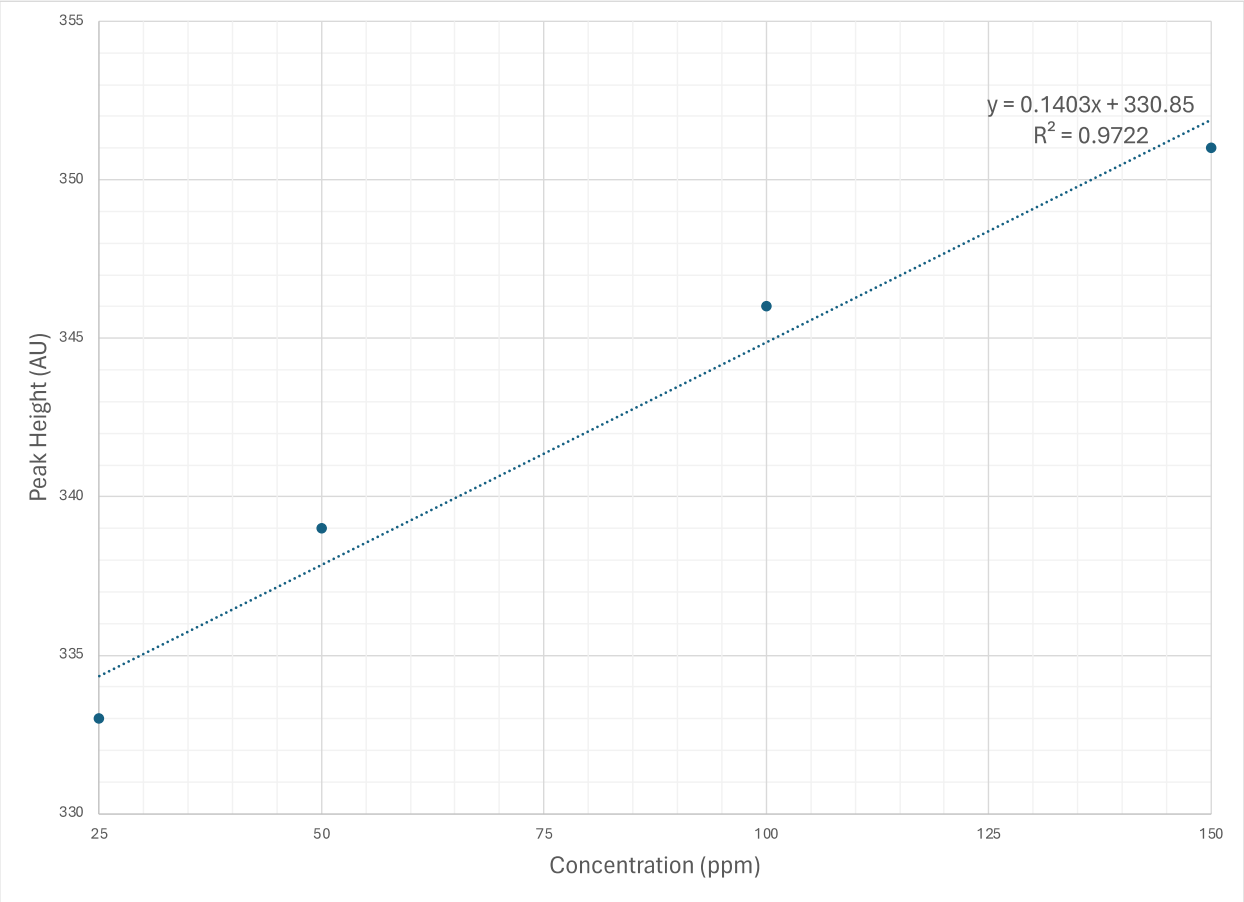

Figure 11: Calibration curve of Nisin Standards of concentrations (150 mg L^{-1}, 100 mg L^{-1}, 50 mg L^{-1}, and 25 mg L^{-1}) using Method B.

3.1.3 Method C (2-minute sample injection, -15 kV switch polarity)

The final method tested in this study consisted of a 2-min sample injection time and -15 kV switch polarity. An increased sample injection time is expected to yield a larger signal as there is more analyte present in the experimental run. Additionally, an increase in voltage of the switch polarity is expected to yield results with a shorter migration time and with higher efficiencies and higher resolution. However, this was not the case in terms of faster analysis. As shown in *Table 1,* the migration time of the experimental runs in method C was the same as the experimental runs in both methods A and B. Nonetheless, the results from this method yielded the strongest signals so far. From Table 1, we can see the values of the signal (peak height) are all close to double that of the results from methods A and B. This shows that this method is much more sensitive than the previous two methods studied.

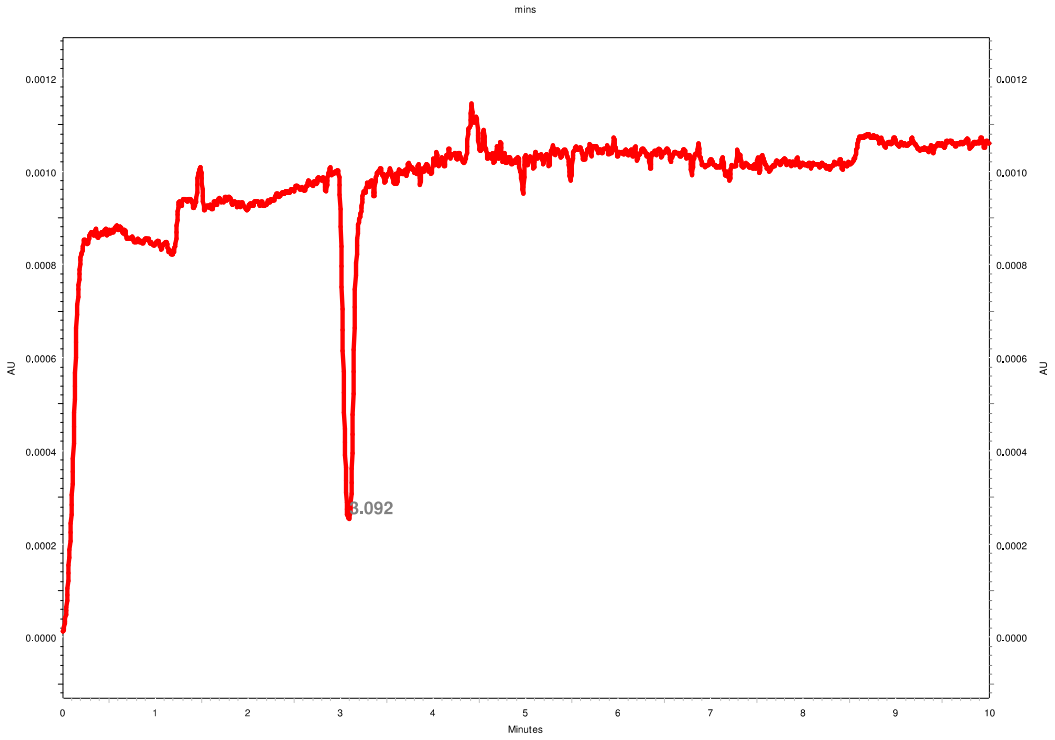

Figure 12: Electropherogram of 25 mg/L nisin standard using method C (peak height 721 AU)

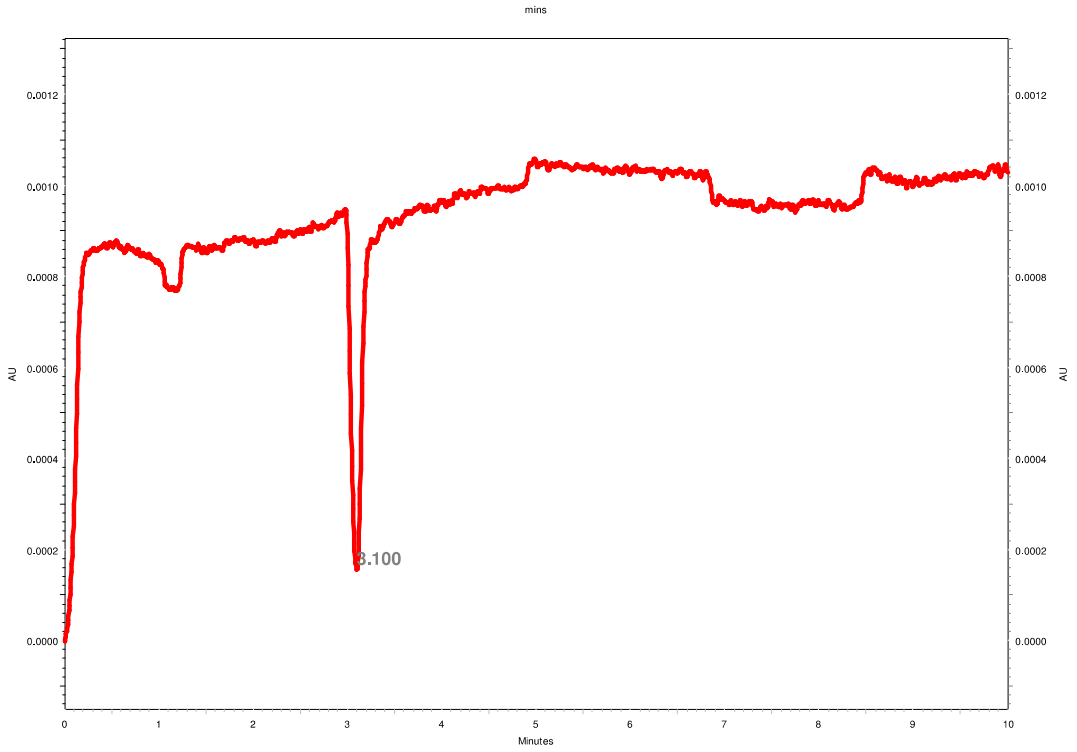

Figure 13: Electropherogram of 50 mg/L nisin standard using method C (peak height 760 AU)

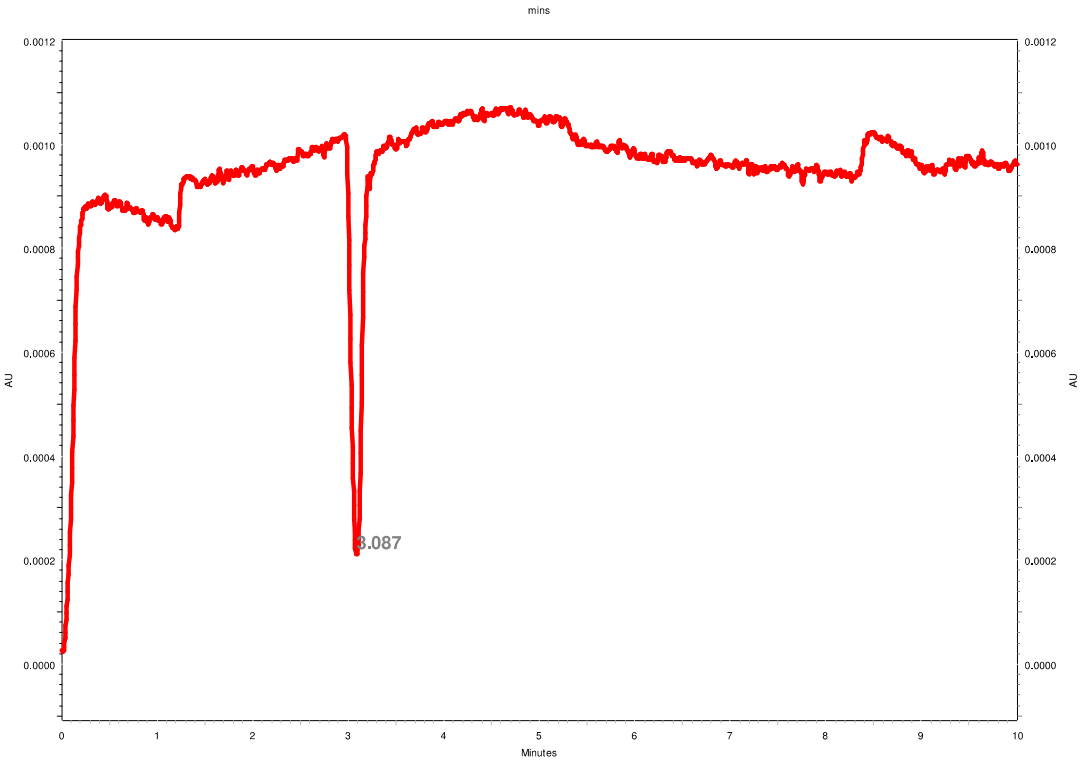

Figure 14: Electropherogram of 100 mg/L nisin standard using method C (peak height 773 AU)

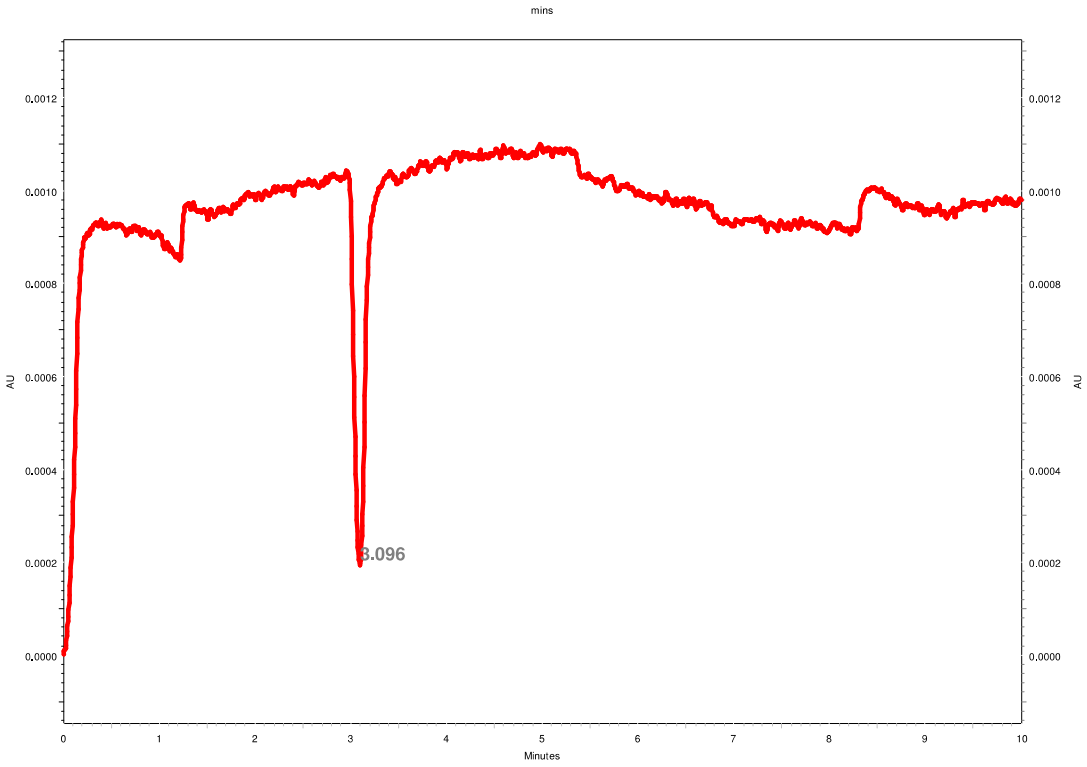

Figure 15: Electropherogram of 150 mg/L nisin standard using method C (peak height 829 AU)

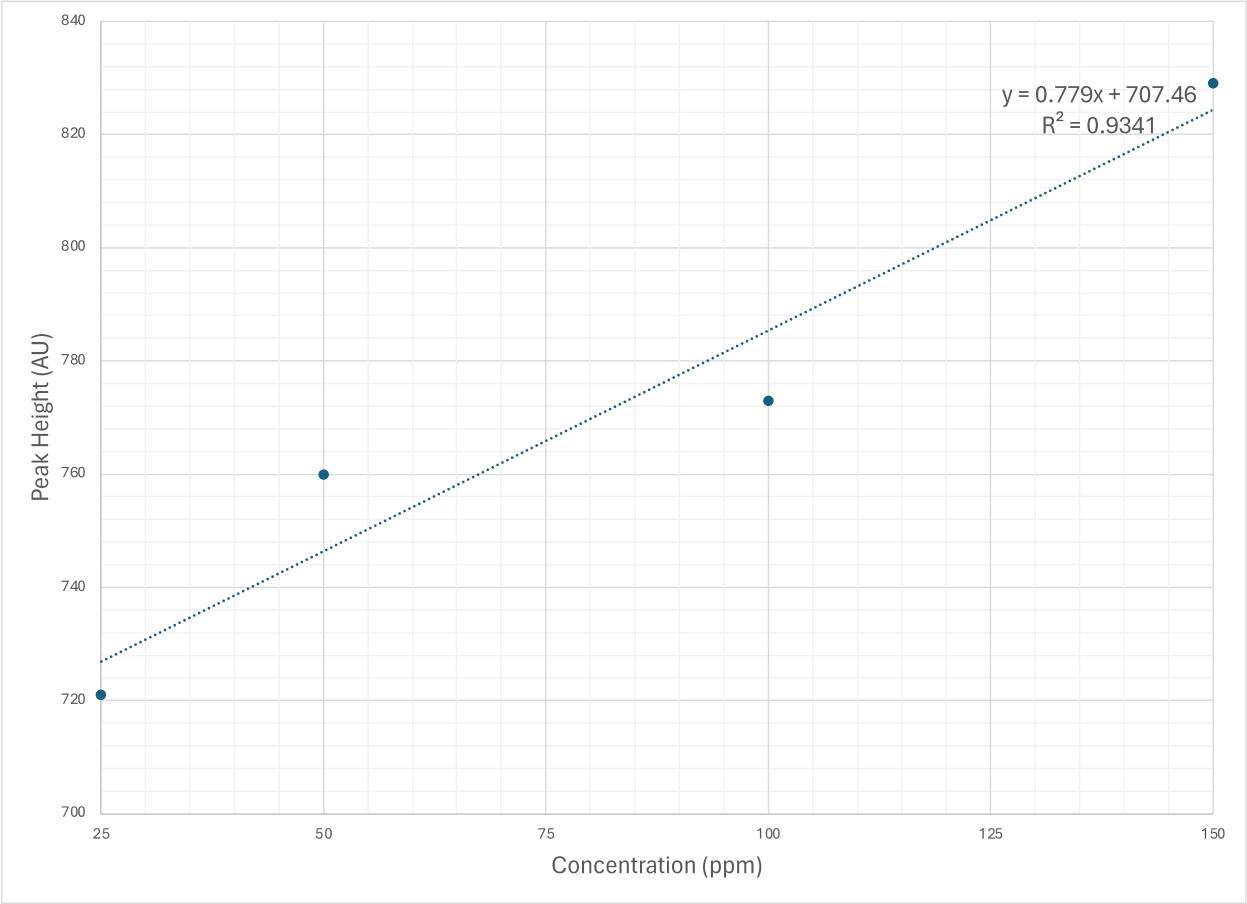

Figure 16: Calibration curve of Nisin Standards of concentrations (150 mg L^{-1}, 100 mg L^{-1}, 50 mg L^{-1}, and 25 mg L^{-1}) using Method C.

3.1.4 Summary

Across the three methods tested, method C showed the greatest sensitivity as shown in *Table 2.* The migration times are all very similar to each other confirming the presence of nisin. The summary of results from the three methods studied are shown below.

	Method A		*Method B*		*Method C*	
Concentration	**Migration Time**	**Peak Height**	**Migration Time**	**Peak Height**	**Migration Time**	**Peak Height**
ppm	**minutes**	**AU**	**minutes**	**AU**	**minutes**	**AU**
25	3.104	339	3.087	333	3.092	721
50	3.096	350	3.096	339	3.100	760
100	3.100	353	3.092	346	3.087	773
150	3.096	376	3.096	351	3.096	829

Table 2: Summary of results comparing the migration time and peak height of nisin standards using methods A, B and C.

3.2 Future Work

In future work, more standards can be run as well as run in duplicate. This would allow for more accurate results and to test reproducibility of methods. Furthermore, a range of different voltages can be tested to see which voltage yields the best results without excessive Joule heating. The effect of sample injection can be further investigated by testing a range of different sample injection times and pressures. Furthermore, testing of nisin in real food samples can be done as the nisin tested in this study was in diluent solutions and not real matrices.

Conclusion

In this study, an LVSS capillary electrophoresis method was partially optimized for the detection of nisin at the parts-per-billion levels in food products. The benefits of this method are fast analysis time, low sample and reagent consumption, and low cost. The optimized method was found to incorporate a 2-min injection time with a -15 kV switch polarity. The method also incorporated a background electrolyte containing 30 mM sodium phosphate dibasic at pH 3.75 with sample solvent composition of 10 mM sodium phosphate dibasic at pH 3.0. In future studies, analysis of multiple switch polarity voltages can be tested as well as different sample times and pressures. Furthermore, method validation needs to be performed to test parameters such as limit of detection (LOD) and limit of quantization (LOQ), linearity, reproducibility, precision, and accuracy.

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