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Research Article

Impact of Acetate, Formate and Lactate Background Electrolytes on Nitrate and Nitrite Separation by Capillary Electrophoresis

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ABSTRACT

This study comparatively evaluated acetate, formate and lactate background electrolytes (30 mM, pH 4.0) for the determination of nitrate and nitrite by capillary electrophoresis. Standard solutions (5, 25 and 50 µg mL^{-1}) were used to investigate the effects of electrolytes on migration time, theoretical plate number, peak symmetry and resolution. Acetate generated the highest electroosmotic flow mobility but resulted in the longest migration, the lowest theoretical plate numbers and pronounced peak fronting. Formate exhibited the highest separation efficiency and better peak symmetry, while lactate produced the shortest migration times at 25 and 50 µg mL^{-1}. Based on these findings, formate and lactate were selected for calibration and accuracy experiments. Both electrolytes demonstrated good linearity (R^2=0.9994 and =0.9999) over the concentration range of 6.25-100 µg mL^{-1}. Lactate provided lower limits of detection, especially for nitrite. Formate revealed a better analytical accuracy, with recoveries of nitrate and nitrite (95.6% and 98.6%, respectively), compared to lactate (79.4% and 72.5%). In conclusion, the most suitable electrolyte is formate for capillary electrophoretic determination of nitrate and nitrite in processed meat products.

Keywords: Resolution, response factor, peak symmetry, capillary electrophoresis, nitrate, nitrite.

1. INTRODUCTION

Nitrate and nitrite are among the most extensively studied anions in foods because of their technological and toxicological properties. In processed meat products, nitrite is used for the purpose of curing, coloring, flavoring, oxidation prevention, and inhibition of *Clostridium botulinum*, whereas nitrate acts as a precursor and is microbiologically reduced to nitrite during processing and storage (Shakil et al., 2022). Despite these functions, the high intake of nitrate and nitrite raises concerns about their potential role in the production of endogenous N-nitroso compounds (Karwowska and Kononiuk, 2020). Therefore, the analysis of nitrate and nitrite is an essential analytical technique for food quality control and regulatory compliance. Among the analytical techniques, capillary electrophoresis (CE) has gained popularity as an alternative approach to nitrate and nitrite determination due to its high separation efficiency, fast analysis time, low reagent consumption, and minimal sample preparation requirements (Voeten et al., 2018). The anionic nature and relatively high electrophoretic mobility of nitrate and nitrite make them suitable analytes for CE using direct UV detection (Farsang et al., 2025). Several CE methods have been described for the determination of nitrate and nitrite in environmental (Farsang et al., 2022), biological and food samples (Kamilova et al., 2023; Rincón et al., 2003). CE separation depends on the interplay between electrophoretic migration and electroosmotic flow (EOF) (Rathore, 2004). The apparent migration velocity of an analyte is determined by the combined contributions of its electrophoretic mobility and EOF. It was also mentioned that effective separation in CE can hardly be accomplished when the EOF is much higher

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than the analyte migration, and that resolution deteriorates under such conditions (Freitag and Hilbrig, 2007). As EOF largely depends on the physicochemical properties of the background electrolyte (BGE), the selection of the buffer is considered one of the crucial factors in method development. The composition of the BGE not only determines the magnitude of EOF but also influences analyte ionization, conductivity, electric field distribution, Joule heating, electromigration dispersion, peak symmetry, and detection sensitivity (Palonen et al., 2003). Therefore, changes in the BGE composition may cause alterations in migration time, theoretical plate number, peak symmetry, and sensitivity. These effects are particularly pronounced for anions, including nitrite and nitrate as their separation is achieved within a relatively narrow mobility range (Merusi et al., 2010). It was previously reported that peak broadening and peak symmetry are highly dependent on the difference between the electrophoretic mobility of the analyte and the BGE co-ion (Gebauer and Boček, 1997). The mobility matching principle implies that better peak shape and efficiency can be reached when the mobility of the BGE co-ion approaches that of the analyte. Thus, the physicochemical properties of the BGE can have a direct effect on analytical performance even under constant pH and buffer concentration conditions.

Monocarboxylate BGE are commonly used in CE analysis due to their good buffering capacity, low UV absorbance, and ability to be detected by UV method (Elbashir et al., 2024; Talu and Diyamandoglu, 2004). Acetate, formate, and lactate were selected as readily available and structurally related monocarboxylates that differ in their pKa, ionic mobility, conductivity, and hydration ability, which may influence their electrophoretic behavior and consequently the separation of nitrate and nitrite. Acid dissociation constants of formic acid (pKa ~3.75), lactic acid (pKa ~3.86), and acetic acid (pKa ~4.76) are different at pH 4.0 but comparative studies specifically addressing the effect of chemically different BGEs in similar concentration and pH condition are rather scarce. In particular, their effects on nitrate and nitrite separation have not been comparatively evaluated while maintaining the same BGE concentration, pH and electrophoretic conditions. Nitrous acid (HNO_2) has a pKa of approximately 3.2-3.3; therefore, at the working pH of 4.0, nitrite is predominantly present in its deprotonated form (NO_2^-). It was hypothesized that monocarboxylate BGEs with different acid-base properties and ionic mobility would create different electrophoretic conditions, resulting in differences in nitrite and nitrate migration behavior, peak symmetry, efficiency, and resolution even under the same pH and concentration. Therefore, the aim of the present study was to perform a comparative analysis of three monocarboxylate BGEs (acetate, formate, and lactate, 30 mM-pH 4.0) for the capillary electrophoretic separation of nitrite and nitrate. The effects of BGEs on migration, peak symmetry, theoretical plate number, and separation performance were investigated using standard solutions at different concentrations. Besides, the calibration characteristics, detection limit, analytical sensitivity, and recovery were further evaluated in a sausage matrix to assess the performance of the selected BGEs under food matrix conditions.

2. EXPERIMENTAL

2.1. Chemicals

Certified nitrate and nitrite standard solutions (1000 µg mL^{-1}, Supelco, Sigma Aldrich Co.) were used. Ultra-pure water was used for preparation of all solutions and was freshly prepared on the day of analysis. A commercially available sausage was purchased from a local market (Hatay, Türkiye) and used as the representative processed meat product. Monocarboxylate BGEs were prepared from 30 mM solutions of acetic, formic and lactic acids. The pH of each solution was adjusted to 4.0 with NaOH. The BGE concentration of 30 mM was selected based on previous CE studies employing 30 mM formate-based electrolytes for the separation of nitrate and nitrite (Aftab et al., 2021; Kamilova et al., 2023). Considering the pKa of HNO_2 (3.2-3.3), nitrite is predominantly present in its anionic form at pH 4.0. Therefore, pH 4 was selected as a common condition to comparatively evaluate the effects of different BGE compositions while maintaining the pH and BGE concentration constant. The final pH was verified using a calibrated pH meter (HI2211, Hanna Instruments, Woonsocket, USA). Prior to use, all solutions were filtered through 0.45 µm syringe filters and degassed for 5 min in an ultrasonic bath (621.06.003, ISOLAB GmbH, Germany).

2.2. Methods

2.2.1. Capillary Electrophoresis Conditions

Nitrate and nitrite were separated using a capillary electrophoresis system (G7100, Agilent Technologies) equipped with a diode-array detector (DAD) and controlled by ChemStation software (Agilent). Separations were performed in an uncoated fused-silica capillary (50 µm i.d., 64.5 cm total length, 56 cm effective length, G1600-61232, Agilent). The electrophoretic conditions were adapted from Aftab et al. (2021) with minor modifications. Before the first analysis, the capillary was subsequently conditioned with 0.1 N NaOH (10 min), ultra-pure water (10 min), and BGE (15 min). Between runs, the capillary was rinsed with 0.1 M NaOH (2 min), ultra-pure water (2 min) and running buffer (4 min). Samples were hydrodynamically injected at 50 mbar for 5 s. Electrophoretic separation was performed at -25 kV

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(negative polarity) and 25 °C. Detection was carried out at 210 nm using a bandwidth of 4 nm, response time of 2 s and data acquisition rate of 2.5 Hz. EOF mobility was determined by the current monitoring method using a conductivity boundary established between 30 and 25 mM BGEs of identical pH. The migration time of the conductivity boundary was obtained from the current-time profile and EOF mobility was calculated according to Huang et al. (1988).

2.2.2. Peak Characteristics

Migration time was defined as the elapsed time between sample injection and analyte detection. The response factor was calculated as the ratio of peak area to its corresponding analyte concentration (µg mL^{-1}). Theoretical plate number (N) and resolution were calculated according to the equations described by Dursun and Demetgül (2025). Peak symmetry was expressed as the ratio of the distance from the peak apex to the leading edge and the distance from the apex to the trailing edge, measured at 10% of the peak's height (Szabó et al., 2025).

2.2.3. Calibration Characteristics

External calibration curves were constructed for nitrate and nitrite using the corresponding BGE over the concentration range of 6.25-100 µg mL^{-1}. Calibration linearity was evaluated by least-squares regression analysis. Limit of detection (LOD) and quantification (LOQ) were calculated according to ICH (2023) guidelines using the standard deviation of the analytical response (σ) and the slope of the corresponding calibration curve (S), where LOD=3.3 σ S^{-1} and LOQ=10 σ S^{-1}. Method accuracy was evaluated by recovery experiments using spiked samples. Prior to analysis, the sausage was homogenized using a laboratory blender. Briefly, 5 g of sausage was transferred into a centrifuge tube, mixed with 20 mL of ultrapure water, homogenized and centrifuged (4000 rpm, 5 min). The supernatant was filtered through a 0.45 µm syringe filter before CE analysis. Prior to CE analysis, the filtered extract was mixed with the corresponding BGE at a 1:1 (v/v) ratio, resulting in a two-fold dilution. Known concentrations of nitrate and nitrite (25 µg mL^{-1}) were added to each sample prior to analysis, and recovery values (%) were calculated by comparing the experimentally determined concentrations with the theoretical spiked concentration (Dursun and Güler, 2023). Non-spiked and spiked samples were analyzed under identical CE conditions using the selected electrolytes (formate and lactate).

2.2.4. Statistical Analysis

Electrophoretic performance parameters, including migration time, theoretical plate number, peak symmetry and resolution were analyzed using two-way factorial analysis of variance, where BGE (Acetate, formate and lactate) and analyte concentration (5, 25 and 50 µg mL^{-1}) were considered fixed factors. Response factors obtained from the calibration experiments and recovery values were evaluated for formate and lactate by using Student's t-test. All statistical analyses and interaction plots were performed by using the SPSS package program (Version 22, IBM Statistics).

3. RESULTS and DISCUSSION

3.1. Peak and Separation Characteristics

The electropherogram of nitrate and nitrite analyzed by CE under varied BGEs was shown in Figure 1, while the corresponding peak characteristics were summarized in Table 1. Although all BGEs were prepared at the same concentration (30 mM) and pH (4.0), changing only the buffers significantly affected the migration, peak symmetry, separation efficiency (theoretical plate number) and resolution of both nitrate and nitrite (P<0.001). Among the investigated BGEs, acetate generated the highest EOF mobility, whereas formate and lactate exhibited significantly lower EOF values (Figure 2). Interestingly, this tendency was opposite to that observed for analyte migration times (Table 1).

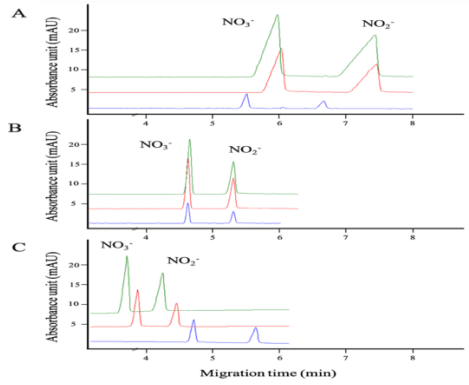


Figure 1. Electropherograms of nitrate and nitrite obtained using three monocarboxylate background electrolytes at different analyte concentrations. (A) Acetate, (B) Formate and (C) Lactate buffers (30 mM, pH 4.0). Blue, red and green traces represent 5, 25 and 50 µg mL^{-1} standard mixtures, respectively.

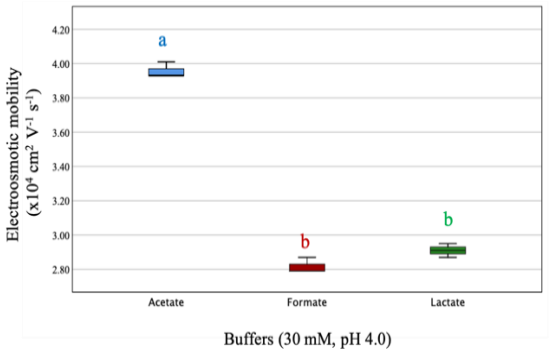


Figure 2. Box plots of electroosmotic mobility obtained using acetate, formate and lactate background electrolytes.

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Overall, acetate produced the longest migration times for both nitrate and nitrite. Lactate resulted in the shortest migration times at 25 and 50 µg mL^{-1}, whereas at 5 µg mL^{-1}, formate produced shorter migration times than lactate for both analytes (Figure 3). This could be explained by the fact that the CE separations were performed under negative polarity, where EOF and anion migration occur in opposite directions (Melanson et al., 2001). Increasing EOF opposes the electrophoretic transport of nitrate and nitrite, resulting in longer apparent migration times. The EOF results were consistent with the acid/base characteristics of the investigated monocarboxylate BGEs.

Table 1. Effect of monocarboxylate background electrolytes and analyte concentration on the electrophoretic performance of nitrate and nitrite determined by capillary electrophoresis.

BGEs	Analyte concentration (µg mL^{-1})	Nitrate			Nitrite			Resolution
		Migration (min)	Symmetry	Plate number	Migration (min)	Symmetry	Plate number	
Acetate	5	5.58±0.10	1.36±0.14	54125±4024	6.77±0.14	1.55±0.14	58742±4834	9.01±0.92
	25	5.96±0.03	3.76±0.06	12627±105	7.37±0.04	5.26±0.13	10211±159	4.00±0.13
	50	5.91±0.02	4.86±0.07	7456±50.2	7.35±0.04	7.01±0.04	5555±7.51	2.95±0.02
Formate	5	4.58±0.03	1.04±0.01	81884±761	5.25±0.03	1.15±0.01	87922±2345	7.96±0.03
	25	4.63±0.00	1.39±0.02	52825±670	5.29±0.00	1.71±0.01	49418±867	5.65±0.09
	50	4.64±0.00	1.47±0.01	48749±388	5.28±0.01	1.76±0.01	46316±158	5.20±0.08
Lactate	5	4.75±0.02	1.21±0.16	69994±13769	5.71±0.03	1.31±0.28	66274±14552	9.39±1.30
	25	3.76±0.05	1.69±0.01	25823±482	4.33±0.07	1.81±0.03	28127±194	4.18±0.08
	50	3.67±0.01	1.97±0.04	19464±520	4.22±0.02	2.34±0.02	17564±367	3.30±0.02
Buffers (A)								
Acetate		5.82^{a}±0.07	3.33^{a}±0.01	24736^{c}±7476	7.16^{a}±0.11	4.61^{a}±0.81	24836^{c}±8616	5.32±0.97
Formate		4.62^{b}±0.01	1.30^{c}±0.07	61152^{a}±5225	5.28^{b}±0.01	1.54^{c}±0.10	61219^{a}±6730	6.27±0.43
Lactate		4.07^{c}±0.17	1.62^{b}±0.12	38427^{b}±8886	4.75^{c}±0.24	1.82^{b}±0.17	37321^{b}±8507	5.62±1.02
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.116
Analyte concentration (B)								
5 µg mL^{-1}		4.97^{a}±0.16	1.20^{c}±0.08	68667^{a}±5776	5.91^{a}±0.23	1.34^{c}±0.11	70979^{a}±6259	8.79^{a}±0.51
25 µg mL^{-1}		4.78^{b}±0.32	2.28^{b}±0.37	30425^{b}±5920	5.66^{b}±0.45	2.93^{b}±0.58	29252^{b}±5672	4.61^{b}±0.27
50 µg mL^{-1}		4.75^{b}±0.32	2.77^{a}±0.53	25223^{b}±6134	5.62^{b}±0.46	3.70^{a}±0.83	23145^{b}±6048	3.82^{b}±0.35
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AxB Interaction		<0.001	<0.001	0.394	<0.001	<0.001	0.720	0.024

BGEs: Background electrolytes. The data were expressed as mean±standard error (n=3). $^{a-c}$ Different superscript letters within each factor indicate significant differences (*P*<0.05).

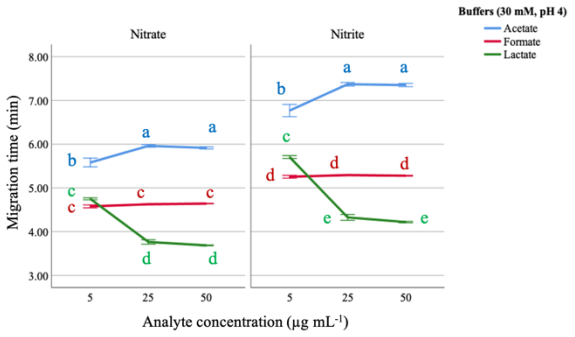


Figure 3. Interactive effect of background electrolyte and analyte concentration on the migration times of nitrate and nitrite. Error bars represent standard error (n=3). Different letters indicate significant differences among the treatment groups (*P*<0.05).

Formate (pKa ~3.75) and lactate (pKa ~3.86) are expected to be largely dissociated at pH 4.0 and produced comparable EOF mobilities. In contrast, acetate (pKa ~4.76) is much less ionized at pH 4.0, resulting in a lower concentration of charged buffer species and consequently a lower ionic strength. The lower ionic strength of the acetate BGE reduces electrostatic screening of the negatively charged capillary surface, which is consistent with the higher EOF mobility observed for acetate. In addition, the lower ionic strength and the resulting conductivity mismatch between the sample zone and the BGE may enhance electromigration dispersion, providing a plausible explanation for the more pronounced peak fronting observed with acetate, particularly at higher analyte concentrations. Aftab et al. (2021) reported migration times of approximately 2.2 min for both nitrate and nitrite using a 30 mM formate at pH 4.0. Also, compared with the present findings, shorter migration times have been reported for nitrate and nitrite using acetate (Shiddiky et al., 2006) and lactate (Pinheiro et al., 2019) BGEs. The differences in instrumental parameters, including effective capillary length, applied voltage and electrophoretic conditions

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used may also have contributed to the observed variation in migration of anions (Dursun and Demetgül, 2025).

Besides migration, the BGE markedly influenced capillary efficiency or theoretical plate number (Table 1). Formate produced the highest theoretical plate numbers for both nitrate and nitrite (61152 and 61219, respectively), while acetate produced the lowest plates (24736 and 24836). Interestingly, the highest efficiencies were obtained with formate despite its relatively low EOF mobility. This result indicates that maximizing EOF alone does not necessarily improve electrophoretic performance. It was also demonstrated that excessively high EOF enhances hydrodynamic dispersion within the capillary, resulting in broader peaks and consequently lower theoretical plate numbers (Ghosal and Chen, 2012). The consistently high plate numbers obtained with formate therefore suggest that this electrolyte provided more favorable electrokinetic matching between the migrating anions and the BGE than acetate or lactate.

Peak symmetry exhibited a similar tendency. Also, peak symmetry was strongly affected by analyte concentration regardless of the BGEs (Table 1). At the lowest analyte concentration (5 µg mL^{-1}), all three BGEs generated nearly symmetrical peaks. However, increasing analyte concentration progressively deteriorated peak symmetry, particularly in acetate (Figure 4). For nitrate, symmetry increased from 1.36 to 4.86, whereas nitrite showed an even greater increase from 1.55 to 7.01 as concentration increased from 5 to 50 µg mL^{-1}. In contrast, formate maintained symmetry factors below 1.8 over the entire concentration range, indicating substantially lower susceptibility to concentration-dependent peak fronting. A negative correlation (r=-0.884) between analyte concentration and tailing factor has previously been reported for chloride anion analyzed by CE (Topaloğlu and Dursun, 2026). It could be stated that acetate was considerably more susceptible to concentration-dependent electromigration dispersion. As analyte concentration increased, conductivity differences between the sample zone and the BGE became more pronounced (Gaš et al., 1997), resulting in greater electric field induced peak distortion. A smaller change observed with formate indicated that this electrolyte better maintained the electrokinetic equilibrium within the capillary, thereby preserving peak symmetry at the studied analyte concentrations.

Although resolution was not significantly affected by BGE (P=0.116), analyte concentration significantly influenced separation quality (Figure 5). Resolution decreased from 8.79 at 5 µg mL^{-1} to 3.82 at 50 µg mL^{-1}, irrespective of BGEs (Table 1). The decrease in resolution with increasing concentration is consistent with the deterioration in peak symmetry and also reduction in theoretical plate number as shown in Table 1. Consistent with the present findings, better resolution was reported for cations with higher theoretical plate number and better symmetry (Dursun and Demetgül, 2025). It should also be noted that all investigated BGEs consistently provided resolution values well above the minimum criteria of 1.5 required for baseline separation (Snow, 2023).

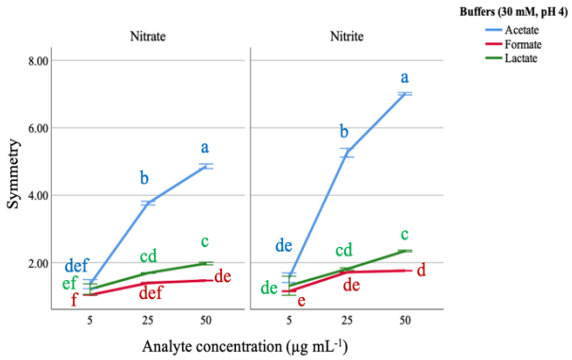


Figure 4. Interactive effect of background electrolyte and analyte concentration on the symmetry of nitrate and nitrite peaks. Error bars represent standard error (n=3). Different letters indicate significant differences among the treatment groups (P<0.05).

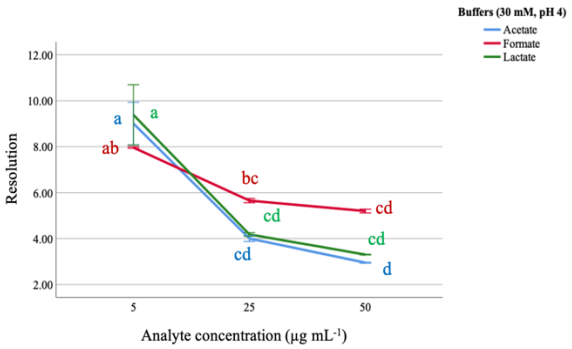


Figure 5. Interactive effect of background electrolyte and analyte concentration on the resolution of nitrate and nitrite. Error bars represent standard error (n=3). Different letters indicate significant differences among the treatment groups (P<0.05).

3.2. Calibration Characteristics

According to the electrophoretic performance findings mentioned above, formate and lactate BGEs were selected for the calibration and method accuracy experiments because their better peak symmetry and separation efficiency compared with acetate. The calibration characteristics of nitrate and nitrite analyzed using formate and lactate BGEs were summarized in Table 2. Higher linearity was achieved for both analytes irrespective of the BGEs, with coefficients of determination (R^2) ranging from 0.9994 to 0.9999 over the concentration range of 6.25-100 µg mL^{-1}. In accordance with the AOAC guidelines, calibration curves with R^2 values exceeding 0.99 demonstrate adequate linearity between instrumental response and analyte concentration (AOAC, 2002).

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Because the response factor was calculated as the ratio of peak area to analyte concentration, it represents the average detector response generated per unit concentration and therefore provides an alternative measure of calibration sensitivity (Dursun and Güler, 2023). The calculated response factors were consistent with the slopes of the corresponding calibration curves. As shown in Table 2, formate and lactate BGEs exhibited similar response factors and calibration slopes for both nitrate and nitrite (Table 2).

Regardless of the BGE, nitrate consistently produced higher response factors than nitrite (Table 2), consistent with the findings of Kamilova et al. (2023). It could be stated that the detector response toward nitrite was more sensitive to the composition of the BGE than that of nitrate because of the lower molar absorptivity of nitrite than nitrate (Fukushi et al., 1999). Although both BGEs exhibited comparable analytical sensitivity, differences in detection capability were observed (Table 2).

Table 2. Calibration characteristics of nitrate and nitrite determined by capillary electrophoresis using formate and lactate background electrolytes.

Background electrolytes	Characteristics	Nitrate	Nitrite
Formate	Response factor[§]	3.82±0.34	3.25±0.12
	Equation (y=a*x*±b)	y=3.28*x*+8.96	y=3.18*x*-0.26
	Linearity (R^2)	0.9995	0.9994
	Limit of detection (µg mL^{-1})	2.97	2.60
	Limit of quantification (µg mL^{-1})	9.00	7.89
Lactate	Response factor[§]	3.66±0.18	3.27±0.04
	Equation (y=a*x*±b)	y=3.26*x*+5.03	y=3.24*x*-0.04
	Linearity (R^2)	0.9996	0.9999
	Limit of detection (µg mL^{-1})	2.55	1.18
	Limit of quantification (µg mL^{-1})	7.72	3.58

Y axis of calibration curve refers to peak area; *x* axis of calibration curve refers to concentration of standard ranged from 6.25 to 100 µg mL^{-1}; a and b are the slope and intercept of calibration curve, respectively. [§]Response factor calculated as the ratio of peak area to corresponding concentration (µg mL^{-1}) was expressed as mean±standard error (n=5). R^2: Determination coefficient.

Lactate provided lower LOD and LOQ values for nitrate (2.55 and 7.72, respectively) and nitrite (1.18 and 3.58) than formate (2.97 and 9.00 for nitrate, 2.60 and 7.89 for nitrite). These limits were comparable with findings of Iammarino et al. (2014), but higher than that of Kamilova et al. (2023) and Della Betta et al. (2016). In most cases where stacking and/or pre-conditioning methods are not used, LOD values for most ions determined by CE ranged from 0.01 to 10.0 µg mL^{-1} (Paull and King, 2003). As the calibration slopes and response factors of the two BGEs were nearly identical, the lower detection limits obtained with lactate are unlikely to be explained by calibration sensitivity alone as stated by Topaloğlu and Dursun (2026).

3.3. Recovery and Method Accuracy

The accuracy of the present method was evaluated by recovery experiments using spiked sausage samples, and the results were summarized in Table 3. The levels of nitrate in non-spiked samples analyzed using formate and lactate BGEs were determined as 4.34 and 4.42 µg mL^{-1}, respectively. However, nitrite was not detected with either BGE. After spiked with 25 µg mL^{-1} of each anion, the formate BGE produced recoveries of 95.6% for nitrate and 98.6% for nitrite, whereas significantly lower recoveries were obtained with lactate (79.4% and 72.5%, respectively). According to AOAC performance guidelines, recoveries within 80-110% are generally considered acceptable for analyte concentrations at ppm level (AOAC, 2002). The recoveries obtained using formate demonstrated better method accuracy, while those obtained using lactate, particularly for nitrite, were below the recommended range. The better recoveries achieved with formate were consistent with the electrophoretic performance observed throughout the present findings. Formate produced higher theoretical plate numbers and better peak symmetry than lactate (Table 1), thereby improving peak integration and quantitative reliability in the sausage matrix.

In contrast, the lower recoveries observed with lactate (Table 3) indicated lower quantitative accuracy in the sausage matrix despite the satisfactory precision of the recovery measurements. This interpretation could be supported by the calibration results, where both electrolytes exhibited comparable linearity and analytical sensitivity, indicating that the reduced recoveries observed with lactate originated primarily from matrix effects during electrophoretic separation. The precision of the recovery results was satisfactory for both BGEs. The RSD values were 1.98% and 6.52% for nitrate and nitrite, respectively, with formate, and 2.63% and 3.55%, respectively, with lactate (Table 3). The lower recoveries obtained with lactate cannot be attributed to poor repeatability of the recovery measurements. Instead, the results indicate a systematic reduction in recovery in the sausage matrix, particularly for nitrite.

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Table 3. Method accuracy evaluated by recovery experiments for nitrate and nitrite determined in sausage samples using formate and lactate background electrolytes.

Background electrolytes	Nitrate	Nitrite
Formate		
Spiked standard (μg mL^{-1})	25	25
Non-spiked sample (μg mL^{-1})	4.34±0.06[§]	nd
Spiked sample (μg mL^{-1})	28.2±0.15	24.7±0.54
Recovery (%)	95.6	98.6
RSD of Recovery (%)	1.98	6.52
CI lower (95%)	94.2	93.7
CI upper (95%)	97.1	103
Lactate		
Spiked standard (μg mL^{-1})	25	25
Non-spiked (μg mL^{-1})	4.42±0.08[§]	nd
Spiked (μg mL^{-1})	24.3±0.15	18.1±0.21
Recovery (%)	79.4	72.5
RSD of Recovery (%)	2.63	3.55
CI lower (95%)	77.8	70.5
CI upper (95%)	81.0	74.4

Spiked and non-spiked samples were expressed as mean±standard error (n=9). [§]Detected above the LOD but below the corresponding LOQ. RSD: Relative standard deviation. CI: 95% confidence interval. nd: not detected.

Yuwono and Indrayanto (2005) also reported that, as the concentration of the analyte decreased from 100% to 1 μg mL^{-1}, a broad range of recoveries (98-102% and 80-110%, respectively) could be considered acceptable, with the RSD increasing from 1.3% to 11%, respectively. These findings demonstrate that although both BGEs performed satisfactorily during calibration with standard solutions, only formate maintained its analytical performance when applied to a complex meat matrix. Matrix performance should also be considered because the physicochemical interactions occurring during electrophoretic separation can substantially influence quantitative accuracy as stated by Topaloğlu and Dursun (2026).

Although recovery correction was applied, the nitrate contents calculated using formate and lactate were not similar. The nitrate concentration determined in the commercial sausage sample was 36.3 mg kg^{-1} using the formate BGE and 44.5 mg kg^{-1} using lactate. These nitrate levels fall within the concentration range commonly reported for processed meat products, where residual nitrate levels generally remain below 100 mg kg^{-1} depending on formulation, curing practice and storage conditions (Karwowska and Kononiuk, 2020). The difference in nitrate concentration calculated using formate and lactate BGEs is most likely associated with the lower recovery obtained with lactate (Table 3), indicating that recovery correction may not have fully compensated for matrix-related effects during electrophoretic separation. A similar observation was also reported by Della Betta et al. (2016), who applied CZE to commercial meat products and found that residual nitrite was not determined in several samples despite the presence of nitrate. This could be attributed to a decrease in nitrite during processing and storage because nitrite reacts with myoglobin, lipids and other meat constituents or is oxidized to nitrate, resulting in the predominance of residual nitrate in the final product (Higuero et al., 2020).

4. CONCLUSION

Differences in the BGE altered EOF, migration, theoretical plate number, peak symmetry and analytical sensitivity, confirming that buffer composition is a critical factor governing electrophoretic performance. The chemical nature of monocarboxylate BGEs significantly influenced the CE determination of nitrate and nitrite under the present experimental conditions. Among the investigated electrolytes, formate provided a better analytical performance by combining lower EOF, higher separation efficiency, improved peak symmetry and higher quantitative accuracy. Although lactate exhibited calibration characteristics comparable to those of formate and even lower LOD values, particularly for nitrite, its substantially lower recoveries in sausage samples indicated poorer quantitative accuracy in the food matrix. These findings emphasize that BGE selection should be based not only on calibration characteristics but also on electrophoretic behavior and matrix performance. Overall, the results indicate that 30 mM formate at pH 4.0 was the most suitable monocarboxylate BGE among those investigated for the capillary electrophoretic determination of nitrate and nitrite under the conditions of the present study. However, the application of the method to a food matrix was limited to a single commercial sausage sample; therefore, broader applicability requires further validation. In addition, the present study evaluated only a single concentration and pH condition for each electrolyte. Further investigations involving different food matrices, as well as different formate concentrations and pH values, are warranted to optimize separation efficiency and sensitivity and to assess method robustness.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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