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Influence of the sample matrix composition on the accuracy of the ion chromatographic determination of anions

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Abstract

The influence of high concentrations of chloride on the accuracy of the determination of nitrite and nitrate by suppressed ion chromatographic analysis was studied using UV–Vis and conductivity detectors. It was found that in the presence of high concentrations of chloride between 0.1 and 10.0 g/l, the retention times of nitrite and nitrate were significantly increased, and co-elution of the two anions was observed at chloride concentrations greater than 15.0 g/l. The nitrite response (absorbance) was found to decrease with increased chloride concentration, whereas the nitrate response did not change significantly. Calibration graphs for nitrite (1–10 mg/l) in solutions containing chloride were linear but their slopes decreased exponentially with increase in chloride concentration. At the same time the slopes of the calibration graphs for nitrate under similar conditions did not vary significantly. It was found that the decrease in the nitrite peak area was the result of a decrease in the absorptivity (210 nm) caused by the protonation of NO_2^- in the suppressor column. The increase in the retention times of the anions can be explained by the self-elution effect of the chloride present in the sample and on-column changes in eluent composition.

Keywords: Matrix effects; Nitrite; Nitrate; Inorganic anions; Chloride; On-column eluent change

1. Introduction

Ion chromatography (IC) is the method of choice for the determination of anionic composition but in the case of a chloride matrix, the determination of some anions (nitrite, nitrate and bromide) becomes almost impossible owing to peak overlapping. This chloride interference can be reduced by the precipitation of silver chloride with silver acetate or silver oxide or by passing the sample through a column containing a cation-exchange resin in the Ag^+ form [1–7]. However,

these methods of chloride removal can result in blockage of the separation column by colloidal silver chloride and coprecipitation of the anions of interest [8].

One possibility to overcome these problems is to use the method of on-column matrix elimination, which was first described by Ito and Sunahara [9] and later exploited by Rokushika et al. [10], Marheni et al. [11] and Brandão et al. [12]. The method involves the conversion of the separation column into an appropriate anion form using an eluent containing the matrix anion. A good separation can be achieved in this way provided that the composition of the eluent is

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optimized, that is, the concentration of the matrix anion in the eluent should be as high as possible to achieve effective on-column matrix elimination but not too high to separate the anions of interest [12]. Studies using on-column matrix elimination for the determination of anions in the presence of a chloride and/or sulphate matrix [10–12] have reported that the benefit of using this method is that no additional influence of the matrix anion on the separation parameters such as retention times and peak areas are observed. In a recent paper by Carozzino and Righini [13], no problems associated with an excess of chloride when using an HPIC AS4A-SC (Dionex) separation column for the determination of anions in seawater were reported.

When analysing samples containing a high concentration of chloride (meat and cheese extracts, seawater) in our laboratory, strong interference by chloride was observed. Since on-column matrix elimination was not successful, e.g., nitrite and nitrate peaks were poorly resolved and broadened when the chloride concentration in the eluent was greater than 50 mM, a detailed study was made to explain above-mentioned phenomena.

2. Experimental

2.1. Chemicals and reagents

All the reagents were of analytical-reagent grade. Stock standard solutions of NO_2^- and NO_3^- (1.000 g/l) were prepared by dissolving 1.499 g of NaNO_2 and 1.371 g of NaNO_3 in 1 l of 18 M Ω /cm water (obtained using a Milli-Q system; Millipore). A stock eluent solution for ion chromatography was also prepared by dissolving 1.908 g of Na_2CO_3 and 1.428 g of NaHCO_3 in 1 l of 18 M Ω /cm water and a working eluent solution was made by adding 20 ml of acetonitrile to 100 ml of the stock eluent solution and diluting with water to 1 l. Stock standard solutions of Cl^- and SO_4^{2-} (50.0 and 25.0 g/l) were prepared by dissolving 82.42 g of NaCl and 36.98 g of Na_2SO_4 in 1 l of 18 M Ω /cm

water. A series of working standard NO_2^- , NO_3^- , Cl^- and SO_4^{2-} solutions were prepared by appropriate dilution of the stock standard solutions.

2.2. Apparatus

Samples were analysed using a Dionex 4000i ion chromatograph fitted with a conductivity detector. The analytical column was an HPIC AS4A-SC protected by an HPIC AG4A-SC guard column. An ASRS-1 self regenerating suppressor, current setting 1, was operated in the chemical suppression mode. All components were obtained from Dionex. The eluent (1.80 mM Na_2CO_3 –1.70 mM NaHCO_3) and regenerant (electrolysed 18 M Ω /cm water) flow-rates were 2.0 and 3 ml/min, respectively. The sample-loop volume was 50 μ l. UV detection at 210 nm and spectra recording were carried out using a SpectraSYSTEM UV 2000 spectrophotometer (Spectra-Physics).

3. Results and discussion

When chromatograms of samples containing increasing concentrations of chloride were compared, an increase in the retention times of nitrite and nitrate and a decrease in the peak area of nitrite were observed. At the same time, no change in the peak area of nitrate or a significant alteration in peak shape occurred. This was surprising because previous studies on the separation of anions in high ionic strength matrices had indicated both a decrease in retention times and peak broadening caused by self-elution and the loss of band-compression effects for the analyte anions [14].

Owing to above-mentioned discrepancy between our and published results, synthetic solutions containing 2.0 mg/l of nitrite and 5.0 mg/l of nitrate in a chloride matrix (0.0–10.0 g/l) were prepared and analysed using HPIC AG4A-SC and HPIC AS4A-SC (Dionex) guard and separation columns with UV-Vis and conductivity detectors connected in series. The retention times of both anions increased with increase in chloride concentration (Fig. 1). Co-elution of

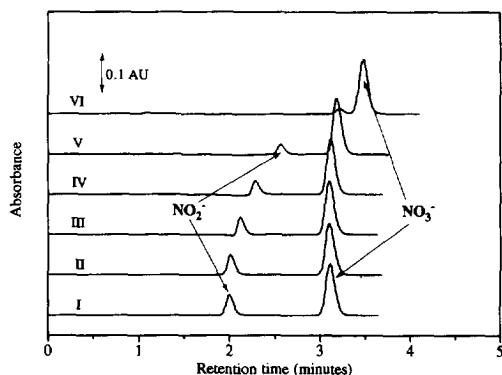


Fig. 1. Chromatograms of model solutions containing 2.0 mg/l of NO_2^- and 5.0 mg/l of NO_3^- in a chloride matrix (I = 0.0, II = 0.1, III = 0.5, IV = 1.0, V = 5.0 and VI = 10.0 g/l). Anions were separated on an HPLC AS4A-SC separation column. Other parameters are described under Experimental.

both species occurred at chloride concentrations higher than 15.0 g/l. The effect of increasing chloride concentration on the nitrite and nitrate

Table 1
Influence of chloride concentration (C_{Cl^-}) on the nitrite and nitrate peak areas

C_{Cl^-} in the sample (g/l)	Nitrite peak area	Nitrate peak area
0.0	618	1899
0.01	619	1898
0.1	561	1885
0.5	480	1928
1	411	1917
2	304	1913
5	154	1915

Table 2
Slopes (S), y -intercepts (A_0) and correlation coefficients (r^2) for the Eq. 1 expressing peak areas for nitrite and nitrate as a function of chloride content (C_{Cl^-})

C_{Cl^-} (g/l)	S (l/mg)	$A_0(\text{NO}_2^-)$	$A_0(\text{NO}_3^-)$	$r^2(\text{NO}_2^-)$	$r^2(\text{NO}_3^-)$
0.0	274.2	-32.4		0.99987	
0.5	176.3	-23.2		0.99996	
1.0	132.2	27.3		0.99964	
2.0	97.0	-26.0		0.99999	
0.0	379.5		-13.7		0.99989
10.0	375.3		+85.2		0.99975

peak areas is shown in Table 1. This confirms our original findings.

3.1. Effect of chloride on the nitrite peak area

The effect of chloride concentration on the peak areas of both anions was studied by recording a set of calibration graphs in which the chloride concentration (matrix) varied between 0.0 and 2.0 g/l and between 0.0 and 10.0 g/l, while the nitrite and nitrate concentrations (at fixed chloride concentration) varied between 0.0 and 10.0 mg/l. Calibration graphs (based on peak areas) obtained with UV-Vis detection exhibit linearity for both analytes and can be expressed by the equation

$$A = SC + A_0 \quad (1)$$

where A represents peak area, S the slope of the calibration graph in l/mg, C the concentration of the analyte (NO_2^- and NO_3^-) in mg/l and A_0 the absorbance at zero concentration (blank). The coefficients from the regression equations for the calibration graphs are summarized in Table 2. The decrease in the slopes (i.e., the sensitivity) of the calibration graphs for nitrite approaches exponentially, at infinite chloride concentration, 32% of the value of the slope of the calibration graph for chloride-free solutions.

Since the oxidation of nitrite to nitrate can be excluded (Table 1), diffusion of HNO_2 through the suppressor membrane into the regenerant stream [15] was investigated. However, the nitrite anion was not detected in the regenerant

even after several repeated injections of a sample containing a high concentration of nitrite (50 mg/l). Because the change in the sensitivity may originate from the transformation of NO_2^- into HNO_2 , UV-Vis spectra of samples containing 2 mg/l NO_2^- in 18 M Ω /cm water and in 0.2 M HCl (pH 0.82) were recorded (Fig. 2). The absorption maximum for nitrous acid is at 202 nm (curve HNO_2), whereas for an aqueous solution of nitrite it is at 210 nm (curve NO_2^-). It was found that the absorptivity of HNO_2 at 210 nm represents 31.5% of that of nitrite in the neutral medium. This is almost the same value as obtained by the limiting value of the sensitivity represented in Table 2, which means that the lowest sensitivity of the nitrite detection at 210 nm corresponds to the absorption of completely protonated nitrite. The fraction of HNO_2 produced on-suppressor depended on the amount of co-eluted Cl^- and Na^+ (Fig. 3), which is converted on-suppressor into H_3O^+ . This suggests that the decrease in the NO_2^- peak area is the result of an increase in the concentration of H_3O^+ . To confirm this, the NO_2^- response was increased by neutralizing the eluent. Samples containing 2 mg/l of NO_2^- , both with and without 1.0 g/l of Cl^- , were analysed. Neutralization was achieved by the postcolumn addition of NaOH at a flow-rate of 0.5 ml/min. The system is shown in the inset of Fig. 4. Curve A represents a chromatogram of a sample containing 2.0

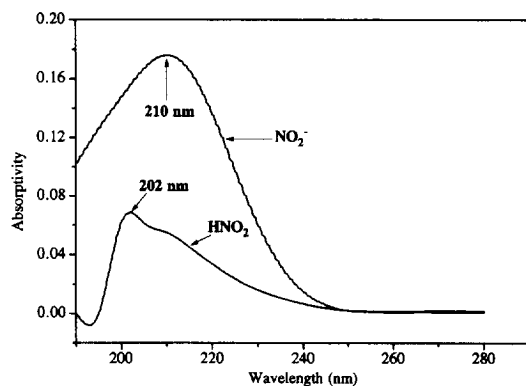


Fig. 2. UV spectra of samples containing 2.0 mg/l of nitrite in 18 M Ω /cm water (NO_2^- curve) and in 0.2 M HCl (HNO_2 curve). Absorption maxima are marked by arrows.

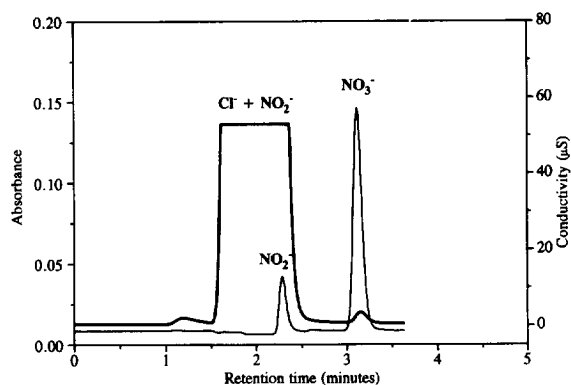


Fig. 3. Chromatogram of a sample containing 2.0 mg/l of NO_2^- , 5.0 mg/l of NO_3^- and 1.0 g/l of Cl^- recorded by UV-Vis (thin line) and conductivity (thick line) detectors. Separation conditions as in Fig. 1.

mg/l of NO_2^- and curve B a sample containing 2.0 mg/l of NO_2^- and 1.0 g/l of Cl^- . In both cases 18 M Ω /cm water was used as the post-column reagent to ensure the same peak broadening/dispersions as when NaOH was added. It is evident from Fig. 4 that the presence of chloride ions causes an increase in the retention time of nitrite and a decrease in peak area. Curve C represents the same sample as curve B, but with 0.1 M NaOH as a postcolumn

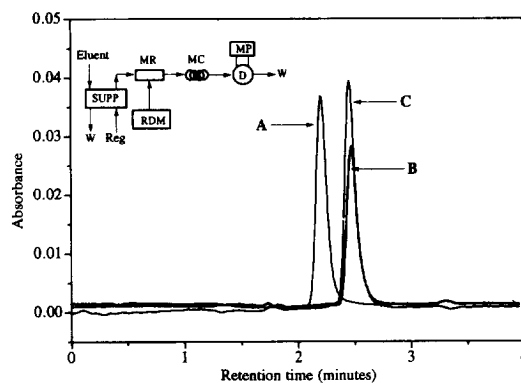


Fig. 4. Chromatograms of samples containing 2.0 mg/l of nitrite in 18 M Ω /cm water (A) and in a chloride matrix (1.0 g/l) (B and C). Postcolumn reagents were 18 M Ω /cm water (A and B) and 0.1 M NaOH (C). In the inset, postcolumn reagent addition is shown schematically. SUPP = suppressor; RDM = reagent-delivery system; MR = membrane reactor; MC = mixing coil; D = detector; MP = data recording system; Reg = reagent; W = waste.

reagent. Evidently neutralization recovers the original height of the nitrite peak, but not its retention time. The slightly higher peak obtained by neutralization with NaOH (curve C) in comparison with the peak represented by curve A indicates that even when a sample containing only nitrite is injected, the nitrite is not completely in the NO_2^- form when leaving the suppressor. Protonation of NO_2^- in the suppressor markedly affects the sensitivity of the UV measurements and conductivity detection (HNO_2 cannot be detected by the conductivity detector). Thus, significant errors may occur when using two-column IC to determine anions with basic properties when the above problems are either overlooked, misinterpreted or ignored by the analyst.

3.2. Effect of chloride on the retention behaviour of anions

The observed increase in the retention times for nitrite and nitrate (Fig. 1) was also unexpected and cannot be explained by the self-elution effect of the matrix anion [9]. Owing to the self-elution process, shorter retention times occurred and therefore additional retention/elution mechanisms were sought. Chromatograms of samples containing nitrite and nitrate in a chloride matrix (15.0 and 50.0 g/l) are shown in Fig. 5. Chromatograms A and B were obtained using the suppressor and C and D were obtained without inclusion of the suppressor in the chromatographic line. If a comparison is made between chromatograms A and B, a self-elution effect of chloride is observed. At a retention time of 1 min, i.e., the system void volume, a very small and a significant detector signal (a on chromatograms A and B) can be observed when 15.0 g/l of Cl^- or 50.0 g/l of Cl^- are present in the sample. The appearance of this peak in chromatogram B could be attributed to the co-elution of nitrite and nitrate in the system void volume as a result of the self-elution effect of a very high concentration of chloride. Regardless of the partial chloride retention by the stationary phase, the Cl^- concentration in the sample plug remains high enough during its transportation

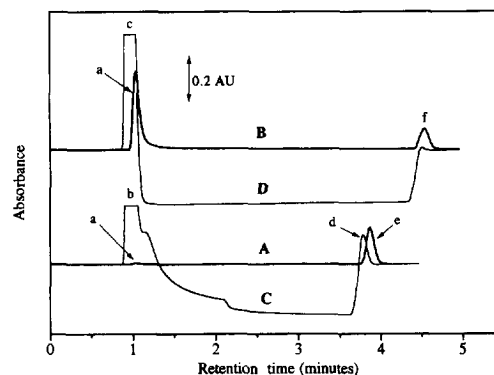


Fig. 5. Chromatogram of a sample containing 2.0 mg/l of NO_2^- and 5.0 mg/l of NO_3^- in a chloride matrix consisting of 15.0 g/l of Cl^- (chromatograms A and C) and 50.0 g/l of Cl^- (chromatograms B and D). C and D, without suppressor; A and B, with suppressor. a, Nitrite and nitrate co-eluted with the system void volume; b and c, displaced carbonate/hydrogencarbonate and co-eluted nitrite and nitrate (c); d and e, co-eluted nitrate and nitrite; f, change in the absorptivity of the eluent.

through the column to prevent nitrite and nitrate being retained by the stationary phase. When samples containing lower Cl^- concentrations (e.g. 15.0 g/l) are injected, chloride ions prevent nitrite and nitrate being retained only for a fraction of the time during which the sample plug moves through the separation column. After the retention of nitrite and nitrate by the stationary phase, the anions are eluted by either the original or modified eluent.

Peak b on chromatogram C corresponds to the displaced carbonate/hydrogencarbonate ions from the stationary phase, and peak c on chromatogram D is the sum of displaced carbonate/hydrogencarbonate, nitrite and nitrate. Peaks d and e (chromatograms A and C) are formed by the co-elution of nitrite and nitrate, while peak f results from the change of the absorptivity of the eluent when its composition is converted from mostly chloride back to the carbonate/hydrogencarbonate form. The negative shift in absorbance in the non-suppressed mode (C and D) is caused by the lower absorptivity of sodium chloride-based eluent with respect to sodium carbonate/hydrogencarbonate-based eluent.

This confirms that self-elution of anions in the presence of high concentrations of chloride and

column conversion from the carbonate/hydrogencarbonate form to the chloride form does occur. Both processes spread through the column with a velocity similar to that of an unretained species. Their duration is always shorter than or equal to the column dead time, t_m (about 1 min under the experimental conditions). Column back-conversion to the initial form starts at the tail of the sample plug. Column back-conversion is finished when the total amount of chloride is eluted. In this case when a sample containing 50 g/l of Cl^- was injected, column back-conversion was completed within ca. 3.5 min (Fig. 5, chromatogram D).

3.3. On-column change of the eluent

The capacity factor k' , for nitrate and nitrite, calculated from the equation

$$k' = \frac{t_s}{t_m} \quad (2)$$

where t_s is the solute retention time and t_m the column dead time, increased when the chloride content in the sample was increased. The capacity factor k' is, according to the equation

$$\log k' = \log C - \log E + \text{constant} \quad (3)$$

proportional to the column capacity C and inversely proportional to the concentration of the eluent E . If it is assumed that the capacity of the column remains constant, then a significant change in chemical composition of the eluent should occur when a sample containing an increased concentration of chloride is injected.

The change in the chemical composition of the eluent may be explained by column back-conversion from the chloride to the carbonate/hydrogencarbonate form. Owing to its stronger affinity the carbonate/hydrogencarbonate eluent replaced completely retained chloride from the stationary phase. The sample plug in the separation column is therefore followed by a modified eluent which is very poor in carbonate/hydrogencarbonate but enriched with chloride. The evidence for this process can be seen in Fig.

5, chromatograms C and D. The broad negative signal which follows peaks b or c on both chromatograms belongs to the modified (chloride-based) eluent. The duration of this negative signal was proportional to the chloride concentration in the sample. It should be noted that the chloride concentration in the modified eluent can never exceed 5.3 mM, which is equivalent (in charge) to the sum of the carbonate/hydrogencarbonate concentration in the original eluent. This process is called "on-column change of the eluent". The reason for the increased retention time of nitrite and nitrate is the weaker elution strength of the modified eluent. From Fig. 3 it is evident that nitrite was eluted with chloride. During the action of the chloride-based eluent, NO_3^- is very slowly exchanged from the stationary phase and its intensive elution continues after the modified chloride-based eluent has been replaced by the original eluent.

To investigate the impact of chloride on the elution efficiency, the dependence of the retention times of nitrite and nitrate on the percentage of carbonate/hydrogencarbonate replaced by chloride was measured. Fig. 6 shows that a significant change in the eluent efficiency occurs when more than 80% of the carbonate/hydro-

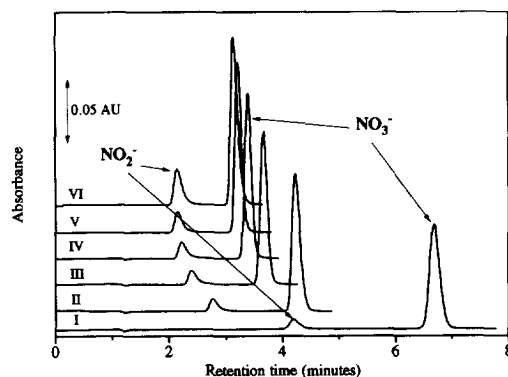


Fig. 6. Chromatograms of a sample containing 2.0 mg/l of NO_2^- (peak a) and 5.0 mg/l of NO_3^- obtained using a modified eluent. Chromatograms I–VI were recorded after replacing 0% (VI), 20% (V), 40% (IV), 60% (III), 80% (II) and 100% (I) of the carbonate/hydrogencarbonate (1.7 mM $\text{HCO}_3^- + 1.8 \text{ mM } \text{CO}_3^{2-}$) eluent with the chloride (5.3 mM) eluent.

gencarbonate is replaced by chloride. This means that samples containing high concentrations of chloride (>0.1 g/l) cause a dynamic on-column change in the eluent which affects the retention processes in the column.

3.4. Verification of the model

The proposed model was verified using two types of synthetic samples, the first containing 5.0 mg/l of bromide in solutions containing increasing concentrations of chloride (from 0.0 to 10.0 g/l) and the second containing 2.0 mg/l of nitrite and 5.0 mg/l of nitrate in solutions containing increasing concentrations of sulphate (from 0.0 to 10.0 g/l).

When the samples containing bromide were injected, an increase in retention time with increasing chloride concentration similar to that of nitrite was observed. This could suggest that the same parameters which affect nitrite also influence the retention behaviour of bromide. However, in contrast to nitrite, the acidification of the eluent zone carrying bromide did not influence its UV absorption and the bromide response remained constant. In a sulphate matrix it was observed that the nitrite and nitrate retention times were shortened proportionally to the increase in concentration of sulphate in the sample. Also the nitrite response remained constant and unaffected by the sulphate content. The reason for the independence of the nitrite peak area of the sulphate concentration can be seen in Fig. 7. Nitrite and sulphate do not co-elute even at 10.0 g/l of SO_4^{2-} and, as a consequence, NO_2^- is not converted into HNO_2 . The carbonate/hydrogencarbonate eluent which follows the sample plug replaced sulphate from the stationary phase very slowly owing to its stronger affinity. Consequently, the carbonate/hydrogencarbonate eluent move through the column faster than the SO_4^{2-} zone. Therefore, unlike in the chloride system, the sample plug is followed by the original eluent and not a modified eluent. This means that the retention of nitrite and nitrate is affected only by the self-elution effect of sulphate.

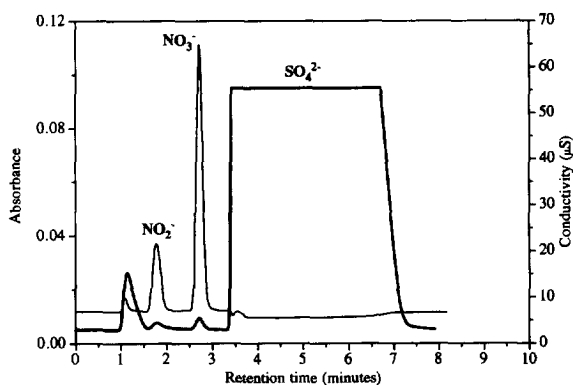


Fig. 7. Chromatogram of a sample containing 2.0 mg/l of NO_2^- and 5.0 mg/l of NO_3^- in a sulphate matrix (10.0 g/l of SO_4^{2-}) recorded by UV-Vis (thin line) and conductivity (thick line) detectors. Separation conditions as in Fig. 1.

4. Conclusions

Under the experimental conditions, co-elution of NO_2^- and chloride occurs at chloride concentrations >0.1 g/l. On-suppressor formation of HNO_2 and consequently a lower absorptivity of HNO_2 at 210 nm in comparison with those of NO_2^- cause a decrease in the nitrite response. The on-suppressor protonation causes the signals from the UV-Vis and conductivity detectors to correspond to the pH-dependent fraction of nitrite, even when samples containing nitrite only are analysed. A decrease in the nitrite peak area may be avoided by postcolumn neutralization of the effluent. Standard addition is recommended for the determination of nitrite in samples containing large amounts of chloride, including extracts of meat and milk products and seawater, whereas nitrate may be determined by the use of a calibration graph.

The increased retention times of nitrite and nitrate were attributed to a combination of two processes, a self-elution process which decreases the retention time and the on-column change of the eluent which prolongs the retention time. The on-column change of the eluent predominates when the matrix anion has an affinity for the stationary phase comparable to the analyte (e.g., chloride, nitrite and nitrate). In the case of

a significantly stronger affinity of the matrix anion (sulphate) shorter retention times of nitrite and nitrate can be explained by the predominant self-elution mechanism.

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