

Simultaneous determination of nitrate, nitrite and phosphate in environmental samples by high performance liquid chromatography with UV detection

Mohau N. Moshoeshoe

Department of Chemistry
University of Botswana, Private Bag 00704
Gaborone, Botswana
mohaumn7@gmail.com

Veronica Obuseng

Department of Chemistry
University of Botswana, Private Bag 00704
Gaborone, Botswana
Obusengv@mopipi.ub.bw

Abstract—An HPLC method using an UV detector was developed for the simultaneous determination of nitrites, nitrates and phosphates for environmental samples. Chromatographic separation was achieved on a Phenomenex Synergi Polar-RP LC column using acetonitrile and acidified water (pH 2.7) at 60:40 v/v as mobile phase, isocratically at a flow rate of 0.800 mL/min. Baseline resolution of all the three analytes was achieved within 3 min. The calibration curves for nitrite, nitrate and phosphate in the range of 1.0 – 50.0 ppm were linear with correlation coefficients of more than 0.999 for all the analytes. The developed method was applied to water samples obtained from the wastewater treatment plant in Gaborone, Botswana. The use of HPLC technique in this study demonstrated its ability to carry out a simultaneous determination of nitrite, nitrate and phosphate in water samples. The treatment plant was found to be functioning within expectations, as it led to the removal of 97.6% nitrite, 88.0% nitrate and 90.9% phosphate from the received wastewater. Nevertheless, the concentrations of the analytes in the effluent tank were found to exceed the limits set by the Water Utilities Corporation. Results of the developed method were comparable with those of the traditional Ion chromatography method, showing accuracy values between 95.22% – 98.04%. The precision of the method for the determination of all analytes was determined by RSD values, all of which were lower than 5%. The method is of low cost, fast, has an easy procedure and avoids the use of many reagents – some of which may be hazardous.

Keywords—simultaneous determination; environmental samples; baseline resolution; nitrite; nitrate; phosphate; wastewater treatment; HPLC; Ion chromatography; low cost method

I. INTRODUCTION

Nitrogen is a vital nutrient for both plants and animals [1]. In the environment, it exists as nitric oxide (NO), nitrite (NO_2^-) and nitrate (NO_3^-). These are ubiquitous within biological, food and environmental samples [2]. In water, NO_3^- occurs both naturally and through external inputs which may include run-off from agricultural soils, soil organic matter as well as livestock and human faecal material [3]. Contamination of

groundwater resources by nitrate is mainly a consequence of anthropogenic activities and uncontrolled discharges from industries, agricultural activities, sewage treatment plants and pit latrines [4].

Contamination of water and food sources by nitrate is a concern as it may result in methemoglobinaemia [5], which may eventually lead to mental retardation [6]. Nitrite may react with amines in human bodies to form nitrosamines, which are said to be carcinogenic [1]. In surface waters, excess nitrates and nitrites lead to algal blooms, resulting in lowered levels of oxygen in the water, leading to eutrophication [7].

Phosphates are of great importance in numerous fields such as in the manufacturing of fertilizers, detergents, beverages and semiconductors [8-10]. However, excessive phosphorus concentration in waters leads to over growth of aquatic plants and algal bloom formation, which ultimately result in the depletion of dissolved oxygen in water. This is then followed by the death and decay of aquatic life and degradation of the water quality [11-13].

It is for the above-mentioned reasons that a simple and sensitive analytical method is desirable for the monitoring and routine analysis of nitrates, nitrites and phosphates, both in water and soil samples.

Several analytical techniques such as electrophoresis [14], potentiometry [15], gas chromatography (GC) [2], ion exchange chromatography (IC) [16], and high-performance liquid chromatography (HPLC) [2], have been applied in the determination of NO_2^- , NO_3^- and PO_4^{3-} in different samples. Many of these techniques (e.g. electrophoresis) suffer from drawbacks such as interferences caused sample matrix [17] and/or lack of reproducibility of migration times which can cause errors in peak identification [18]. Although electroanalytical techniques such as potentiometry, amperometry, voltammetry and polarography do not suffer from interferences and do not require extensive pre-treatments [19], they however tend to suffer from poor reproducibility upon reuse [15] due to poisoning of the biosensor films used in

them. GC cannot be used in the determination of PO_4^{3-} due to its requirement that the sample be highly volatile [20,21].

IC is normally the method of choice for the determination of these nutrients. Examples include the analysis of water (natural, waste and seawater) [16], sediments [22], fertilizers and soils [23], foodstuffs [23], dairy products [24] and clinical fluids [25]. Although IC is characterized by high sensitivity, it is prone to temperature variations and matrix effects – particularly in the determination of nitrate in the presence of chlorides (Cl^-), sulphate (SO_4^{2-}) and bromide (Br^-) [26,27]. Modifications to try and circumvent this problem resulted in lower sensitivities and poor resolution between the analyte and interferents peaks [28]. In order to circumvent these difficulties, extensive sample preparations and separation steps are needed, which usually lead to increased overall determination time and low sample throughput [5,24].

HPLC is a much more rapid, sensitive and selective technique than most methods of analysis, hence it is a better choice for the determination of nitrate, nitrite and phosphate in water samples. Although the determination of NO_2^- , NO_3^- , and PO_4^{3-} has been carried out using HPLC, the simultaneous determination of these analytes are limited. The objective of this study was therefore to develop a specific, sensitive, cost-effective, and reliable analytical method that can be applied in the simultaneous determination of nitrate, nitrite and phosphate. The attributes of the developed method (e.g. precision, accuracy, LOD etc.) were determined by comparing HPLC results with those obtained from the IC method.

II. MATERIALS AND METHODS

All commercially available reagents and solvents (potassium dihydrogen phosphate, sodium nitrite, sodium nitrate, potassium thiocyanate, methanol, acetonitrile Dionex, seven-anion standard solution, ECG III KOH eluent generator) used in this study were of analytical-reagent grade and were used without further purification. Distilled, de-ionized water (DDW, 18 M Ω /cm) was used in all sample preparation procedures. All glassware used was soaked in 10% HCl for 24hrs, and rinsed several times with DDW prior to use.

Different mobile phase compositions in various mixture ratios using methanol (Sigma-Aldrich, Steinheim, Germany), acetonitrile (Sigma-Aldrich, Steinheim, Germany) and distilled – deionised water, were used to elute the prepared analyte standards.

A Dionex, seven-anion standard mixture (purchased from Thermo Fisher Scientific, California, USA), which contains a mixture of F (20ppm), Cl^- (100 ppm), NO_2^- (100 ppm), Br^- (100 ppm), NO_3^- (100 ppm), PO_4^{3-} (200 ppm) and SO_4^{2-} (100 ppm) was used to prepare calibration mixtures for nitrate, nitrite and phosphate within a working range of 1.00 to 100.00ppm in DDW. Working standards were prepared freshly daily in 50 mL volumetric flasks.

A. Sample collection

Wastewater samples were collected from the Glenvalley Wastewater Treatment Plant (GVWTP) in Gaborone, Botswana on the 24th Feb 2015. Samples were collected from

the treatment plant at the inlet, primary settlement tank, secondary settlement tank, aeration tank and final effluent. They were immediately taken to the laboratory and stored at 4°C until further analysis.

III. EXPERIMENTAL

A. HPLC Method Optimisation

Analyses with HPLC-UV were performed using an Agilent Technologies HPLC-UV equipped with a diode array detector. In order to achieve the best method characteristics, the main parameters leading to a good separation in HPLC (mobile phase composition, temperature, pH, flow rate, etc) were optimized.

The stationary phase used was a Phenomenex Synergi Polar-RP LC column, with the dimensions: 150 x 4.6 mm 4 μm , 80 Å. This column showed maximized retention and selectivity for polar analytes with an improved peak shape [29]. Furthermore, it is able to withstand low pH conditions, has the highest cation selectivity [29] and is reported to be stable in 100% aqueous conditions [29-31], allowing for a wide range of mobile phase composition variation.

The wavelength of maximum absorbance for all the analytes was determined with an Evolution 201 UV-Vis spectrophotometer (Thermo Scientific, USA), set to scan peaks at $\lambda = 190\text{nm}$ to 800.0nm. A mixture of standard solutions of NaNO_3 , NaNO_2 and Na_3PO_4 was then made, in which the concentration of each of the analytes was 20ppm each. This mixture was also scanned using the same UV-VIS spectrophotometer at a wavelength range of 190 – 800nm. For all the analytes of interest, λ_{max} was found to be 207.4nm. This wavelength was then used for all subsequent experiments using an Agilent Technologies HPLC-UV equipped with a diode array detector.

Different mobile phase compositions in various mixture ratios using methanol, acetonitrile and DDW, were used to elute the prepared analyte standards. Experiments were carried out using methanol, acetonitrile and water at various methanol:water and acetonitrile:water ratios (from 100:0 v/v to 0:100 v/v each). A mixture of the analytes (in which the final concentration of each analyte was 20ppm) was injected at these mobile phase compositions in order to investigate and optimize the separation.

The pH of the mobile phase was altered by adding concentrated H_2SO_4 to the aqueous part of the mobile phase so as to vary the pH of the aqueous part in the range of 0.5 to 6.5, at increments of 0.5 units. A mixture of the analytes (20ppm each) was injected at each pH value in order to investigate and optimize the separation of the different analytes.

System temperature was varied from 10°C up to 70°C, at increments of 5°C for experiments carried out using both methanol and acetonitrile as organic modifiers.

Optimization of the mobile phase volumetric flow rate was carried out in the range of 0.20 mL/min to 1.50 mL/min using the optimum conditions found above. The best separation conditions, hence the optimum mobile phase flow rate, was determined based on the resolution between the different

components at each flow rate. The total analysis time was also taken into consideration.

B. Ion Chromatography (IC) Analysis

A Dionex ICS-2100 Ion Chromatography System and Chromeleon® Chromatography Data System software were used in this study. The chromatography system was fitted with a 2 x 250 mm IonPac AS18 Analytical column, preceded by a 2 x 50 mm IonPac AG18 Guard Column. The EGC III KOH Cartridge was used as an eluent source, set to produce an eluent of 32 mM potassium hydroxide. The column temperature was set to 30°C and the flow rate set at 0.25 mL/min. Detection was carried out using a DS6 heated conductivity cell.

IV. RESULTS AND DISCUSSION

A. HPLC method optimisation

Fig 1 to Fig 3 show the chromatograms which were obtained using a 20ppm mixture of nitrate, nitrite and PO_4^{3-} standards at an optimum wavelength of 210nm. Peak letters correspond to the following substances: a) NO_2^- , b) NO_3^- , c) SCN^- (internal standard) and d) PO_4^{3-} .

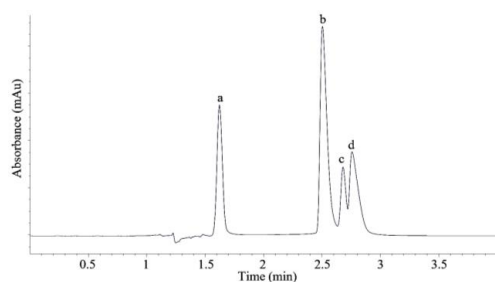


Fig. 1. Chromatogram for a mixture of NO_2^- , NO_3^- , PO_4^{3-} and SCN^- (20ppm each) standards by HPLC-UV using 100 % acetonitrile at a wavelength of 210nm.

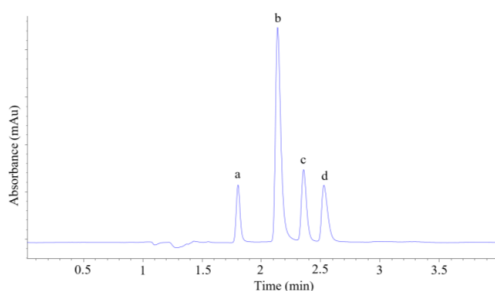


Fig. 2. Chromatogram obtained in the separation of NO_2^- , NO_3^- , KSCN and PO_4^{3-} (at a final concentration of 20ppm each) with a mobile phase composed of CH_3CN : acidified H_2O = 55:45 v/v. Peak letters correspond to the following substances: a) NO_2^- , b) NO_3^- , c) KSCN and d) PO_4^{3-} .

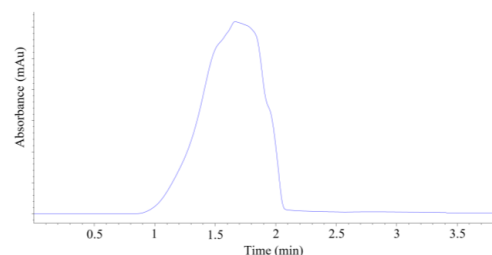


Fig. 3. Chromatogram obtained in the determination of a mixture of NO_2^- , NO_3^- , and PO_4^{3-} standards (at a final concentration of 20ppm each) by HPLC-UV using 100 % water

Elution was improved by increasing the amount of the aqueous component (CH_3CN : acidified H_2O = 55:45 v/v). This resulted in a better resolution between all the three analytes (Fig 2). An additional increase in the polarity of the mobile phase (CH_3OH : acidified H_2O = 50:50 v/v) resulted in loss of resolution even though there was a slight improvement in the shapes of some peaks. Further loss of resolution resulted when the mobile phase was made more polar (Fig 3).

Since a high throughput and productivity was also required, the time of analysis (i.e. method run-time) was also considered. A method with a short run-time saves time and expense, hence it is preferred. Baseline resolution of all the three analytes was achieved at the shortest possible time (3 min; Fig 2) using a mobile phase composition of MeCN : acidified H_2O (pH 2.7) = 55:45 v/v. This is a huge improvement when compared to the ion chromatography method, which has a total run time of more than 10 minutes (see Fig 5).

B. Analytical parameters

The developed HPLC method was validated by determining its figures of merit (accuracy, precision, linearity, limits of detection (LOD) and quantification (LOQ)). The results obtained from the developed method were comparable with those from the traditional IC method. Table 4 shows the numerical values of the concentrations obtained by the two methods.

The accuracy of the HPLC method was expressed through recovery calculations using wastewater samples that had previously been analysed and found to contain 33.87, 20.87 and 5.77ppm NO_2^- , NO_3^- , and PO_4^{3-} respectively. Three different concentrations of each analyte were prepared by measuring 2.5 mL of the wastewater sample in three different volumetric flasks, then spiking with different amounts of 1000 ppm stock solution (of each analyte) and making up to 10mL final volume with DDW. This formed final concentrations of 10ppm, 50ppm and 100ppm each of NO_2^- , NO_3^- , and PO_4^{3-} in the different volumetric flasks. The blank was prepared by spiking DDW with the same amount of analytes to give the same final concentration in the different levels. These spiked samples were then analysed with both HPLC and Ion Chromatography. Percentage recoveries were calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Determined concentration of spiked sample}}{\text{Known spike added concentration}} \times 100\% \quad (1)$$

As shown in Table 1, the recoveries for all samples at all levels were found to be above 93.20%. The high level of accuracy, together with low RSD values (which were found to range from 1.29% (NO_2^-) to 4.92% (PO_4^{3-})) indicate that the method is accurate and precise – hence reliable and its results can be trusted.

Linearity was assessed based on a plot of the analyte peak area ratios to IS (KSCN) versus analyte concentration. The results proved to be linear and acceptable, as the correlation coefficients (R^2) were greater than 0.999 for all the analytes (Table 2).

Linear Dynamic Range (LDR) was determined over the concentration range of 0.200–200 mg/mL, which is a significant progress when compared with most methods for the analysis of nitrate and nitrite, which are linear over only very narrow concentration ranges. These wide linearity ranges enabled quantification of the analytes at different process points.

TABLE 1: RECOVERIES OF NITRITE, NITRATE AND PHOSPHATE SPIKED INTO WASTEWATER SAMPLES

Analyte	Technique	%Recovery at Concentration Level		
		5 ppm	50 ppm	150 ppm
NO_2^-	HPLC	92.08 ± 7.46	94.29 ± 0.13	100.75 ± 1.33
	Ion Chrom	98.76 ± 0.16	98.13 ± 1.12	102.03 ± 3.02
NO_3^-	HPLC	110.65 ± 2.72	92.27 ± 1.20	96.59 ± 1.22
	Ion Chrom	98.89 ± 0.69	97.65 ± 0.36	93.93 ± 0.71
PO_4^{3-}	HPLC	94.25 ± 2.39	88.47 ± 0.17	98.87 ± 0.40
	Ion Chrom	98.52 ± 0.63	93.12 ± 0.21	96.76 ± 0.20

TABLE 2: LINEARITY DATA OBTAINED IN THE ANALYSIS OF NO_2^- , NO_3^- AND PO_4^{3-} USING THE DEVELOPED HPLC-UV/DAD METHOD

Analyte	Calibration equation	R^2	LDR (ppm)
Nitrite	$y = 0.0608x + 0.0050$	0.9995	0.200 – 200.000
Nitrate	$y = 0.3188x + 0.1280$	0.9996	0.200 – 200.000
Phosphate	$y = 0.0956x + 0.0269$	0.9991	0.200 – 200.000

TABLE 3: LOD AND LOQ VALUES OF HPLC AND ION CHROMATOGRAPHY FOR THE ANALYTES OF INTEREST. ALL VALUES ARE GIVEN IN mg/L

Analyte	Nitrite		Nitrate		Phosphate	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
HPLC	0.451	1.504	9.451	31.504	1.334	4.447
Ion Chrom	0.147	0.491	0.340	1.134	0.264	0.880

The calibration sensitivity, defined by IUPAC [32] as the slope of the calibration curve is also reported in Table 2. The high sensitivity values obtained for the analytes imply that low injection volumes will be adequate for detection.

The limits of detections (LODs) and Limits of quantifications (LOQ) were estimated based on the signal background noise. The level of noise was measured from chromatograms of the lowest standard at concentration of 0.01mg/L. The LODs and LOQs were calculated from equations 3 and 4 respectively.

$$\text{LOD} = y_B + 3s_B \quad (n=7) \quad (2)$$

$$\text{LOQ} = y_B + 10s_B \quad (n=7) \quad (3)$$

Where: y_B = blank signal, and s_B = standard deviations of the blank samples spiked with low concentration of analytes.

The LODs obtained by the HPLC-UV/DAD method are given in Table 3.

These values are considerably higher than the LODs obtained by IC and the limits established by WHO [33]. Nevertheless, they are lower than the typical concentrations of these analytes in wastewater samples, therefore the HPLC method is still valid for analysis of this type of samples.

C. Application of method to wastewater samples

Wastewater entering the GVVWTP contains considerable amounts of nitrates, nitrites and phosphates due to the chemicals used in both households and industries. The purpose of the treatment plant is to reduce the amount of these analytes as the water goes through the different treatment stages.

The selectivity of the method was established by the analysis of water samples obtained from the inlet of the wastewater treatment plant. These are the dirtier samples as they have not yet undergone any treatment. No interferent peaks were observed at the retention times of NO_2^- , NO_3^- and PO_4^{3-} in any of the samples (Fig 4). A chromatogram obtained from the analysis of the same sample by Ion Chromatography is shown in Fig 5.

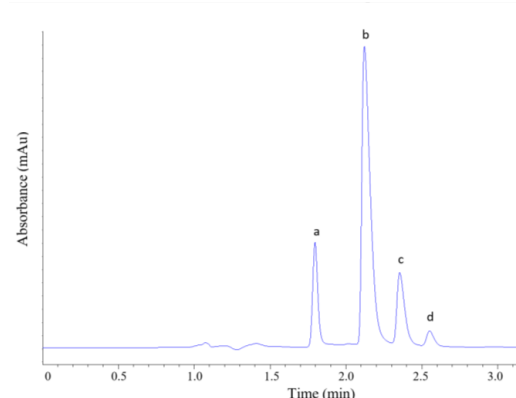


Fig. 4. HPLC-UV chromatogram of raw wastewater sample collected from the inlet of the wastewater purification plant. Peak letters correspond to the following substances: a) NO_2^- , b) NO_3^- , c) SCN^- and d) PO_4^{3-}

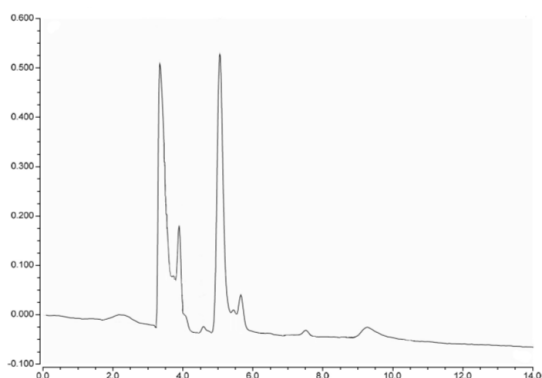


Fig. 5. Ion Chrom chromatogram of raw wastewater sample collected from the inlet of the wastewater purification plant. Peak letters correspond to the following substances: a) F^- , b) Cl^- , c) NO_2^- , d) Br^- , e) SO_4^{2-} , f) NO_3^- and g) PO_4^{3-}

Since Ion chromatography is not selective to the analytes of interest, it is prone to interferences which result from matrix components. More often than not, it was difficult to intergrate the nitrite peak quantitatively due to the interference caused by chloride. As a result, analysis by Ion chromatography requires more sample cleanup steps to remove the interfering species. This increases the overall analysis time and sample handling steps, which may lead to sample losses and/or the introduction of artifacts. method was validated by determining its figures of merit (accuracy, precision, linearity, limits of detection (LOD) and quantification (LOQ)). The results obtained from the developed method were comparable with those from the traditional IC method. Table 4 shows the numerical values of the concentrations obtained by the two methods.

IC was however found to be more precise than HPLC. Table 4 shows the standard deviation and %RSD values obtained in the determination of NO_2^- , NO_3^- and PO_4^{3-} in wastewater tanks. Standard deviation values and %RSD (ion chromatography) range from 0.44 – 3.62, while %RSD (HPLC) range from 1.29 – 4.85.

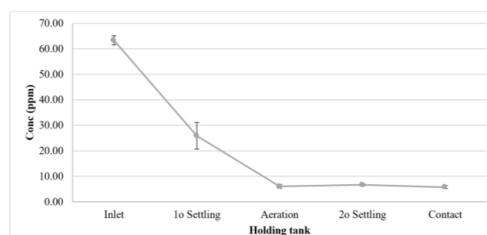
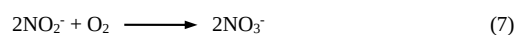
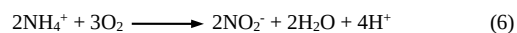
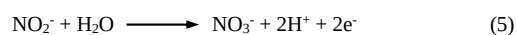
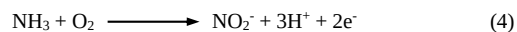


Fig 6. Concentration profile of phosphate along the different holding tanks in the GVWTP at a mobile phase composition range of MeCN:H₂O = 60:40, flowrate of 0.800 mL/min and a column temperature of 30°C.

The effects of treatment process on the amount of PO_4^{3-} is shown in Fig 6.

The water obtained from the effluent tank (at the end of the treatment process) was found to have 9.11% of the amount of phosphate initially present at the inlet. This translates to a phosphate removal efficiency of 90.89%. Similar trends were observed for nitrites and nitrates, with a removal efficiency of 97.59% and 87.99% respectively (see Table 5).

The removal of nitrogen in the water occurs through nitrification – a process in which ammonia is biologically converted to nitrate according to the following equations [34, 35]:



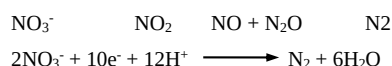
The production of both NO_2^- and NO_3^- in the steps above could be used to explain the trends seen in Fig 2 and Fig 3, whereby the amounts of both NO_2^- and NO_3^- increase from the inlet tank to the primary settlement tank. This process is followed by denitrification, where nitrate is reduced to nitrogen gas [36]:

TABLE 4: COMPARISON OF THE CONCENTRATION OF NO_2^- , NO_3^- AND PO_4^{3-} IN WASTEWATER SAMPLES COLLECTED FROM HOLDING TANKS USING IC AND HPLC –UV METHOD.

Analyte	Technique	Inlet		1° Settlement		Aeration		2° Settlement		Effluent	
		Conc (mg/L) ± Std Dev	% RSD	Conc (mg/L) ± Std Dev	% RSD	Conc (mg/L) ± Std Dev	% RSD	Conc (mg/L) ± Std Dev	% RSD	Conc (mg/L) ± Std Dev	% RSD
NO ₂ -	HPLC	1409.06 ± 63.41	4.50	3528.52 ± 107.27	3.04	115.78 ± 3.80	3.28	143.90 ± 1.85	1.29	33.87 ± 0.76	2.24
	Ion Chrom	1315.41 ± 39.60	3.01	3763.82 ± 26.84	0.71	120.03 ± 1.02	0.85	147.11 ± 1.70	1.16	36.49 ± 1.32	3.62
NO ₃ -	HPLC	173.75 ± 6.17	3.55	340.64 ± 11.01	3.23	21.96 ± 0.45	2.05	15.48 ± 0.63	4.07	20.87 ± 0.71	3.40
	Ion Chrom	157.89 ± 0.69	0.44	348.84 ± 11.79	3.38	23.93 ± 0.71	2.97	16.90 ± 0.24	1.42	22.50 ± 0.75	3.33
PO ₄ 3-	HPLC	63.34 ± 1.79	2.83	25.93 ± 0.53	2.04	6.08 ± 0.17	2.80	6.68 ± 0.22	3.29	5.77 ± 0.28	4.85
	Ion Chrom	63.52 ± 0.63	0.99	23.12 ± 0.21	0.91	6.76 ± 0.20	2.96	6.77 ± 0.15	2.22	5.46 ± 0.10	1.83

TABLE 5: OBTAINED AMOUNTS AND MAXIMUM LIMITS FOR WASTEWATER ENTERING AND LEAVING GVWTP

Holding tank	NO ₂ ⁻ (mg/L)		NO ₃ ⁻ (mg/L)		PO ₄ ³⁻ (mg/L)	
	Obtained amount	WUC limit	Obtained amount	WUC limit	Obtained amount	WUC limit
Inlet	1409.06	200	173.75	100	63.34	30
Effluent (contact tank)	33.87	1.5	20.87	10	5.77	1.5
Removal Efficiency	97.59	--	87.99	--	90.89	--



It is due to the denitrification process that the amounts of NO₂⁻ and NO₃⁻ are reduced as the water travels to the secondary settling tank, aeration tank and contact tanks.

The removal of phosphate in the wastewater is also seen to be effective since the phosphate amount decreases as the wastewater travels through the treatment process. Phosphorus in wastewater can be removed biologically by polyphosphate-accumulating organisms, which accumulate large quantities of phosphate within their cells [37,38]. Chemical removal of phosphorus in wastewater also occurs through precipitation with iron, alum or lime [39].

Although the removal efficiency of these nutrients is satisfactorily high, the amounts of the analytes in the effluent were still above the limits set by the Water Utilities Company as shown in Table 6. This is in contrast to the studies done in 2005 by Nkegbe and co workers [40], who found the effluents to be below the maximum allowable effluent levels of <1 (PO₄³⁻) and <5 (NO₃⁻), thereby implying that the wastewater treatment process is not functioning accordingly in the removal of the analytes of interest from wastewater. This water however undergoes further natural treatment as the effluent from the plant is released to maturation ponds and then discharged to the Notwane River. Some of the water is used to irrigate golf courses, lawns and crops grown under the Glen Valley irrigation project [41].

V. CONCLUSIONS

The use of HPLC technique in this study demonstrated its benefit of being able to carry out a simultaneous determination of NO₂⁻, NO₃⁻ and PO₄³⁻ in water samples. Furthermore, HPLC is fast and does not require extensive sample preparations, which may lead to sample losses and the introduction of artifacts. It also does not require derivatization, hence avoids the use of many reagents – some of which may be hazardous. Furthermore, HPLC is efficient and economical as it requires the use of fewer amounts of reagents. A single injection in HPLC requires filling a 20 µL loop. In contrast, IC requires a sample volume of 5 mL, all of which is consumed in a single injection.

The developed method was employed to quantify nitrite, nitrate and phosphate content of water from different stages of a wastewater treatment plant. The analyte concentrations were found to be reducing at each stage of treatment, with percentage removal of 97.59% (NO₂⁻), 87.99% (NO₃⁻) and 90.89% (PO₄³⁻).

Although the final effluent has not been tested for the presence/absence of pathogenic organisms, it is however environmentally friendly with regards to the analytes of interest and may be used for applications which do not require potable water (such as irrigational or industrial purposes). Although this may not directly augment existing water sources, it may provide an additional source of water supply which will assist in meeting the water needs of Botswana.

REFERENCES

- [1] Chetty, A. A. & Prasad, S. 2009. Flow injection analysis of nitrate-N determination in root vegetables: Study of the effects of cooking. *Food Chemistry*, **116**:561–566.
- [2] Akyüz, M. & Ata, S. 2009. Determination of low level nitrite and nitrate in biological, food and environmental samples by gas chromatography–mass spectrometry and liquid chromatography with fluorescence detection. *Talanta*, **79**:900–904.
- [3] Mikuška, P. & Vecera, Z. 2003. Simultaneous determination of nitrite and nitrate in water by chemiluminescent flow-injection analysis. *Analytica Chimica Acta*, **495**:225–232.
- [4] Stamatis, G., Alexakis, D., Gamvroula, D. & Migros, G. 2011. Groundwater quality assessment in Oropos-Kalamos basin, Attica, Greece. *Environmental Earth Sciences*, **64**:973–988.
- [5] Burakham, R. Oshima, M., Grudpan K. & Motomizu S. 2004. Simple flow-injection system for the simultaneous determination of nitrite and nitrate in water samples. *Talanta*, **64**:1259–1265.
- [6] Cemek, M. Akkaya, L., Birdane, Y. O., Seyrek, K., Bulut, S. & Konuk, M. 2007. Nitrate and nitrite levels in fruit and natural mineral waters marketed in western Turkey. *Journal of Food Composition and Analysis*, **20**:236–240.
- [7] Lyddy-Meaney, A. J., Ellis, P. S., Worsfold, P. J., Butler, E. C. V. & McKelvie, I. D. 2002. A compact flow injection analysis system for surface mapping of phosphate in marine waters. *Talanta*, **58**:1043–1053.
- [8] Motomizu, S & Li, Z. H. 2005. Trace and ultratrace analysis methods for the determination of phosphorus by flow-injection techniques. *Talanta*, **66**:332–340.
- [9] Hrynyszyn, P., Jastrzębska, A. & Szlyk, E. 2010. Determination of phosphate compounds in meat products by 31-Phosphorus Nuclear Magnetic Resonance spectroscopy with methylenediphosphonic acid after alkaline extraction. *Analytica Chimica Acta*, **673**:73–78.
- [10] Welz, B., Lepri, F. G., Araujo, R. G. O., Ferreira, S. L. C., Huang, M., Okrus, M. & Becker-Ross, H. 2007. Determination of phosphorus, sulfur and the halogens using high-temperature molecular absorption spectrometry in flames and furnaces – A review. *Analytica Chimica Acta*, **647**:137–148.
- [11] Ejhieh, A. N. & Masoudipour, N. 2010. Application of a new potentiometric method for determination of phosphate based on a surfactant-modified zeolite carbon-paste electrode (SMZ-CPE). *Analytica Chimica Acta*, **658**:68–74.
- [12] Nagendrappa, G. Mahadevaiah, M. S., Kumar, Y., Mansour, S., Galil, A., Suresha, M. S. & Sathish, M. A. 2007. A Simple Spectrophotometric Determination of Phosphate in Sugarcane Juices, Water and Detergent Samples. *E-Journal of Chemistry*, **4**:467–473.
- [13] Shyla, B., Mahadevaiah & Nagendrappa, G. 2011. A simple spectrophotometric method for the determination of phosphate in soil, detergents, water, bone and food samples through the formation of

- phosphomolybdate complex followed by its reduction with thiourea. *Spectrochimica Acta Part A*, **78**:497–502.
- [14] Connolly, D. & Paull, B. 2001. Rapid determination of nitrate and nitrite in drinking water samples using ion-interaction liquid chromatography. *Analytica Chimica Acta*, **441**:53–62.
- [15] Sohail, M. & Adeboju, S. B. 2008. Electroimmobilization of nitrate reductase and nicotinamide adenine dinucleotide into polypyrrole films for potentiometric detection of nitrate. *Sensors and Actuators B*, **133**:333–339.
- [16] Thabano, J. R. E., Abong'o, D. & Sawula G. M. 2004. Determination of nitrate by suppressed ion chromatography after copperised-cadmium column reduction. *Journal of Chromatography A*, **1045**:153–159.
- [17] Alonso, M.C.B. & Prego, R. 2000. Determination of silicate, simultaneously with other nutrients (nitrite, nitrate and phosphate), in river waters by capillary electrophoresis. *Analytica Chimica Acta*, **416**:21–27.
- [18] Francois, C., Morin, Ph. & Dreux, M. 1995. Effect of the concentration of 18-crown-6 added to the electrolyte upon the separation of ammonium, alkali and alkaline-earth cations by capillary electrophoresis. *Journal of Chromatography A*, **706**:535–553.
- [19] Cosnier, S., Da Silva, S., Shan, D. & Gorgy, K. 2008. Electrochemical nitrate biosensor based on poly(pyrrole-viologen) film–nitrate reductase–clay composite. *Bioelectrochemistry*, **74**:47–51.
- [20] Helmke, S.M. & Duncan, M.W. Measurement of the NO metabolites, nitrite and nitrate, in human biological fluids by GC–MS. 2007. *Journal of Chromatography B*, **851**:83–92.
- [21] Liu, Z. & Rochfort, S. 2013. A fast liquid chromatography–mass spectrometry (LC–MS) method for quantification of major polar metabolites in plants. *Journal of Chromatography B*, **912**:8–15.
- [22] Colina, M., Ledo, H., Gutiérrez, E., Villalobos, E. & Marín, J. 1996. Determination of total phosphorus in sediments by means of high pressure bombs and ion chromatography. *Journal of Chromatography A*, **739**:223–227.
- [23] Stalikas, C. D., Konidari, C. N. & Nanos, C. G. 2003. Ion chromatographic method for the simultaneous determination of nitrite and nitrate by post-column indirect fluorescence detection. *Journal of Chromatography A*, **1002**:237–241.
- [24] Gapper, L. W., Fong, B. Y., Otter, D. E., Indyk, H. E. & Woollard, D. C. 2004. Determination of nitrite and nitrate in dairy products by ion exchange LC with spectrophotometric detection. *International Dairy Journal*, **14**:881–887.
- [25] Helaleh, M. I. H. & Korenaga, T. 2000. Ion chromatographic method for simultaneous determination of nitrate and nitrite in human saliva. *Journal of Chromatography B*, **744**: 433–437.
- [26] Ito, K., Takayama, Y., Makabea, N., Mitsui, R. & Hirokawa, T. 2005. Ion chromatography for determination of nitrite and nitrate in seawater using monolithic ODS columns. *Journal of Chromatography A*, **1083**:63–67.
- [27] Dahllöf, I., Svensson, O. & Torstensson, C. 1997. Optimising the determination of nitrate and phosphate in sea water with ion chromatography using experimental design. *Journal of Chromatography A*, **771**:163–168.
- [28] Rozan, T. F. & Luther III, G.W. 2002. An anion chromatography/ultraviolet detection method to determine nitrite, nitrate, and sulfide concentrations in saline (pore) waters. *Marine Chemistry*, **77**:1–6.
- [29] Synergi Full Range Selectivity for Reversed Phase Separation.pdf. In: <https://phenomenex.blob.core.windows.net/documents/9df83c96-26ac-4df6-9b61-1c381e13cf8c.pdf>. Accessed on 24 January 2015.
- [30] Whelan, T. J., Gray M. J., Slonecker, P. J., Shalliker, R. A. & Wilson, M. A. 2005. Study of the selectivity of reversed-phase columns for the separation of polycarboxylic acids and polyphenol compounds. *Journal of Chromatography A*, **1097**:148–156.
- [31] Orioli, M., Aldini, G., Beretta, G., Facino, R. M., Carini, M. 2005. LC–ESI-MS/MS determination of 4-hydroxy-trans-2-nonenal Michael adducts with cysteine and histidine-containing peptides as early markers of oxidative stress in excitable tissues. *Journal of Chromatography B*, **827**:109–118.
- [32] Currie, L. A. 1999. Nomenclature in evaluation of analytical methods including detection and quantification capabilities¹: (IUPAC Recommendations 1995). *Analytica Chimica Acta*, **391**:105–126.
- [33] WHO 2011. Guidelines for drinking-water quality, 4th edition. World Health Organization. In http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/ Accessed on 03 March 2015
- [34] EPA. 2002. Nitrification. In http://www.epa.gov/safewater/disinfection/tcr/regulation_revisions.html. Accessed on 26 Aug 2014
- [35] Strous, M., Van Gerven, E., Zheng, P., Kuenen J. G. & Jetten, M.S.M. 1997. Ammonium Removal from Concentrated Waste Streams with The Anaerobic Ammonium Oxidation (Anammox) Process in Different Reactor Configurations. *Water Research* **31**:1955–1962
- [36] Knowles, R. 1982. Denitrification. *Microbiological Reviews* **46**: 43–70
- [37] Kishida, N., Kim, J., Tsuneda S. & Sudob, R. 2006. Anaerobic/oxic/anoxic granular sludge process as an effective nutrient removal process utilizing denitrifying polyphosphate-accumulating organisms. *Water Research*, **40**:2303–2310.
- [38] Oehmen, A. Saunders A. M., Vives, T. M., Yuan, Z. & Keller J. 2006. Competition between polyphosphate and glycogen accumulating organisms in enhanced biological phosphorus removal systems with acetate and propionate as carbon sources. *Journal of Biotechnology*, **123**:22–32.
- [39] de-Bashan, L.E. & Bashan, Y. 2004. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). *Water Research*, **38**:4222–4246.
- [40] Nkegbe, E., Emongor, V. and Koorapetsi, I. 2005. Assessment of Effluent Quality at Glen Valley Wastewater Treatment Plant. *Journal of Applied Sciences*, **5**:647–650.
- [41] Monyamane, Y. 2011. Risk Analysis of Wastewater Use in Crop Production: A Case of Glen Valley Irrigation Scheme, Botswana. MSc Thesis in IWRM. University of Zimbabwe. Faculty of Engineering. pp 3–10