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Novel HPLC Technique for Determining NO_2-N and NO_3-N Levels in Water as an Alternative to SKALAR Cadmium Reduction Method

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Abstract

A novel technique was developed on High Performance Liquid Chromatography (HPLC) instrument that features a photodiode Array UV detector to determine the nitrite-nitrogen and nitrate-nitrogen levels at Qurryah Seawater Treatment Plant located along the Arabian Gulf seawater, as well as in waste water and groundwater in the eastern province of the Kingdom of Saudi Arabia at very low concentrations. The primary objective of this research is to identify a cost-effective alternative that is sufficiently accurate for measuring trace levels of nutrients in water, serving as a replacement for the traditional SKALAR cadmium reduction method used for quantifying nitrite-nitrogen and nitrate-nitrogen levels in water. The HPLC instrument utilized in this study was the 8998 Photodiode Array detector from Waters Alliance e2695. A Phenomenex Luna C-18 HPLC Column (5 µm, 250 × 4.6 mm) at an operating temperature of 40˚C was employed for the separation of analytes. The prepared aqueous solutions included solvent A (washing) at a 50:50 v/v% ratio of $CH_3OH:H_2O$, solvent B (seal wash) at a 10:90 v/v% ratio of $CH_3OH:H_2O$, and solvent C (needle wash) at a 75:25 v/v% ratio of Acetonitrile (ACN):H_2O, while solvent D, which represents the mobile phase, consisted of a 55:45 v/v% mixture of Acetonitrile (ACN):H_2O at a flow rate of 1.0 mL/min. These conditions ultimately facilitated optimal analyte elution and separation. Acidified deionized water with a pH of 2.5 was filtered through a 0.22 µm membrane, and the resulting deionized water filtrate was used to prepare the aqueous solution for solvent D (mobile phase) at a 55:45 v/v% ratio of ACN:H_2O. The mobile phase was maintained at a flow rate of 1.0 mL/min, with an injection volume of 20 µL in the method. The results obtained from the proposed HPLC method were found to be comparable to those from the traditional SKALAR Cadmium reduction method (ASTM D3867), which was conducted using a continuous flow analyzer for determining trace levels of nitrate and nitrite-nitrogen in water. High-Performance Liquid Chromatography (HPLC) consistently shows a lower percentage of Relative Standard Deviations (%RSD) when analyzing QA NO_3-N samples, achieving a %RSD of 1.32% compared to the 2.45% %RSD recorded with the SKALAR instrument. For QA NO_2-N samples, HPLC has proven to have a %RSD of 1.74%, while the SKALAR method produced a significantly higher %RSD of 4.58%. This study concludes that the HPLC instrument is cost-effective, offering an estimated annual savings of USD 20,000, while also being dependable, facilitating rapid analysis, and ensuring a fast turnaround time. It allows for increased throughputs, requires minimal maintenance, conserve water resources, safer, and does not create cadmium-contaminated wastes. When looking at the SKALAR instrument, HPLC is considered a more cost-effective and environmentally friendly choice.

Keywords

Nitrite-Nitrate Nitrogen, High-Performance Liquid Chromatography (HPLC), UV PDA, SKALAR, Cadmium Reduction Method, Arabian Gulf Treatment Seawater (QWSTP)

1. Introduction

The Southern Area Laboratories Division, which is accountable to the SAP Technical Support Department, is responsible for the ongoing monitoring and assessment of different environmental parameters of groundwater, waste-water and the Qurryah Seawater Treatment Plant (QSWTP). The samples of nitrite-nitrate-N are consistently gathered from the intake of QSWTP, along with the two discharge points located along the Arabian Gulf Coast, as depicted in **Figure 1**. The research detailed in this study draws inspiration from earlier work that helped establish the foundation for optimizing the essential HPLC parameters necessary for mobile phase optimization and UV detection. This work was originally conducted by SHIN-SHOU CHOU, JEN-CHIEN CHUNG, and DENG-FWU HWANG (2003) at the Department of Food Science, Taiwan Ocean University, with additional contributions from Mohau N. Moshoeshoe and Veronica Obuseng (2018) from the Department of Chemistry and Chemical Technology at the National University of Lesotho, as well as the Department of Chemistry at the University of Botswana [1] [2].

This paper focuses on the two different methods used for measuring the trace levels of nitrite-nitrate-nitrogen in seawater. This type of analysis is essential for assessing the nutrient concentrations in seawater to prevent any extension of water pollution, maintain the health of aquatic ecosystems and biodiversity, and for various industrial applications, including corrosion management. This paper examines the viability of the newly developed method utilizing HPLC in replacement of SKALAR cadmium reduction method to measure the concentrations of

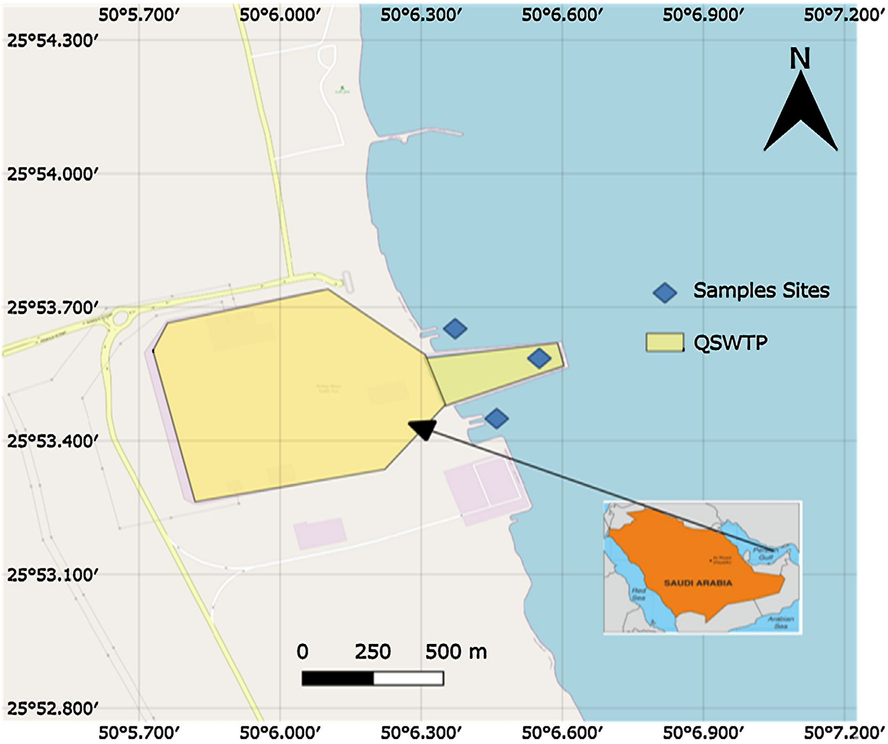


Figure 1. QSWTP map showing the three sample sites.

nitrite-nitrogen and nitrate-nitrogen in seawater, waste water, and groundwater at extremely low levels. A simple filtration process was employed for easy analysis, utilizing a 0.22 μm membrane within the Cole-Parmer Vacuum Filtration Holder Assembly. The ultimate goal of this method was to find the best ideal parameters for the developed HPLC method that can be exploited to accurately measure the trace levels of nitrate-nitrogen and nitrate-nitrogen in seawater, drinking water and effluent water.

There are various methodologies used in laboratories for determining simultaneous measure of both nitrite nitrate-nitrogen in water, yet some of them such as spectroscopic technique normally suffers from interreferences and its inability to detect low levels of these nutrients. The SKALAR method, which is also referred to as the wet chemistry continuous flow analyzer, is presently the approved standard methodology utilized by the Saudi Aramco SAP Technical Support Department—Southern Area Laboratories Division within the Analytical Support Unit—Environmental Lab Segment to quantify the concentrations of nitrite-nitrogen and nitrate-nitrogen in seawater, waste water and groundwater. The SKALAR instrument operates on the principle of converting nitrate into nitrite through a cadmium reduction technique, which is subsequently followed by a colorimetric analysis of nitrite. This analysis utilizes diazotization and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride. This method is widely used and approved by various regulatory agencies. However, this method has many drawbacks as it consumes large quantities of water, requires frequent costly preventive maintenance which includes the regular replacement of high expensive consumable parts such as tubes, auto-sampler needle, fragile column containing granulated cadmium to reduce nitrate to nitrite, and membrane filters every max 3 months. Needles to mention the deleterious impacts due to the cadmium and copper by-products that continuously flow into the water sink. The new developed method on HPLC; however, was found safer, easier to implement and cost effective compared to the traditional cadmium reduction method as it does not consume much of water, does not require preparation of hazardous reagents or require highly expertise personnel to operate and troubleshoot.

2. Literature Review

The Arabian Gulf can be described as a unique body of water, distinguished by its shallow depth, semi-enclosed nature, limited precipitation, and low buffering capacity. These characteristics contribute to its specific features, such as heightened salinity and temperature fluctuations. The average depth of the Arabian Gulf is approximately 35 meters, and its semi-enclosed shape restricts water exchange with the broader ocean. The surrounding arid climate leads to minimal rainfall, which impacts freshwater input and results in increased salinity levels. The lack of freshwater and dissolved minerals in the Gulf limits its ability to neutralize pH changes, making it susceptible to pollution and alterations in water chemistry. As a result, these factors create several notable characteristics, including: 1) Elevated Salinity: The Gulf is known for its high salinity, with Total Dissolved Solids (TDS) exceeding 43,800 mg/l, significantly higher than typical seawater. 2) Temperature Variations: Summer temperatures can rise above 36˚C, while winter temperatures may drop below 15˚C. 3) Nutrient Enrichment: The Gulf experiences increased nutrient enrichment, partly due to anthropogenic activities related to urban expansion and the weakening of winter Shamal winds, which contribute to deoxygenation and the onset of hypoxia in certain areas. 4) Desalination: Given the limited availability of freshwater, desalination is a primary source of drinking water in the Gulf region, although it also presents environmental challenges related to the discharge of waste brine (Saeed *et al.*, 2019) [3]. For these reasons, Saudi Aramco, in accordance with established domestic environmental laws and signed international treaties, is dedicated to reducing environmental impact, complying with local & international regulations, fostering circular economy and environmental sustainability. Accordingly, Saudi Aramco has established a comprehensive environmental management system (Saudi Aramco, 2022) [4].

As cited in The Arabian Sun (2021), Aramco referred to the Master Gas System (MGS) as “the most ambitious energy project in history” in 1976. Unmatched in both scale and expense, the initial MGS was completed in 1982 and had the capacity to process approximately 3.5 billion standard cubic feet of gas daily, which is equivalent to the energy produced by 750,000 barrels of crude oil. The associated gas that was previously utilized to sustain pressure in oil reservoirs was now designated for industrial applications. As an alternative solution to enhance efficiency in maintaining reservoir pressure, Saudi Aramco adopted a new method: the injection of treated seawater. The Qurayyah Sea Water Treatment Plant, a significant project, commenced operations in 1978, pumping 3.7 million barrels of seawater per day from the Arabian Gulf. Coast. This facility effectively eliminated impurities, though it did not remove salt. Extensive pipelines, designed to endure the corrosive nature of the Gulf's highly saline water, transported the treated seawater to injection sites within the Ghawar field. Aramco's seawater injection system supplanted the previous practice of utilizing water sourced from onshore saline aquifers for the maintenance of reservoir pressure [5].

in the Arabian Gulf, the levels of surface water nitrate are predominantly low, varying from 0 to 0.16 μM. Similarly, nitrite concentrations are generally minimal, with average values approximately at 0.06 μg/L, as reported by a study conducted at Khalifa University. Nonetheless, elevated nitrate concentrations may be detected in certain locations, particularly adjacent to the Iranian waters, where they can attain levels of 3.25 μM. Furthermore, nitrate concentrations tend to rise with increasing depth, with the most significant levels recorded in the northern part of the Arabian Gulf (Ismail & Al Shehhi, 2022) [6].

According to Chou *et al.* (2003), a range of analytical techniques has been established for the quantification of nitrate and nitrite, which have been utilized in the examination of water, and various other matrices. These techniques encompass spectrophotometry, High Performance Liquid Chromatography (HPLC), ion chromatography (IC), gas chromatography (GC), polarographic methods, and capillary electrophoresis (CE). Traditionally, spectrophotometric techniques have been employed to measure nitrate and nitrite levels in water; however, their limited sensitivity for detecting trace amounts of these analytes may lead to unreliable results due to interferences from the sample matrix. Capillary electrophoresis represents a novel separation technique that offers significant advantages, including rapid simultaneous detection of a broad range of anions, minimal sample requirements, and reduced buffer consumption. Over the last ten years, several methods utilizing HPLC have been developed, which is typically noted for their speed, accuracy, and sensitivity, surpassing those of traditional spectrophotometric methods.

3. Material and Method

3.1. Instrumentation

High Performance Liquid Chromatography (HPLC) instrument equipped with 8998 Photodiode Array detector from Waters Alliance e2695 was employed in this study. Phenomenex Luna C-18 HPLC Column (5 μm, 250 × 4.6 mm) at operating temperature of 40˚C was utilized for the analyte separation. Thermo Scientific Orion StarTM A211 pH Benchtop Meter was used to measure the pH of the mobile phase. Membranes from MILLIPORE, GS type, with pore size of 0.22 μm from Bedford, Massachusetts and filtration set were applied to meet the water grade specifications suitable for HPLC instrument.

3.2. Chemicals

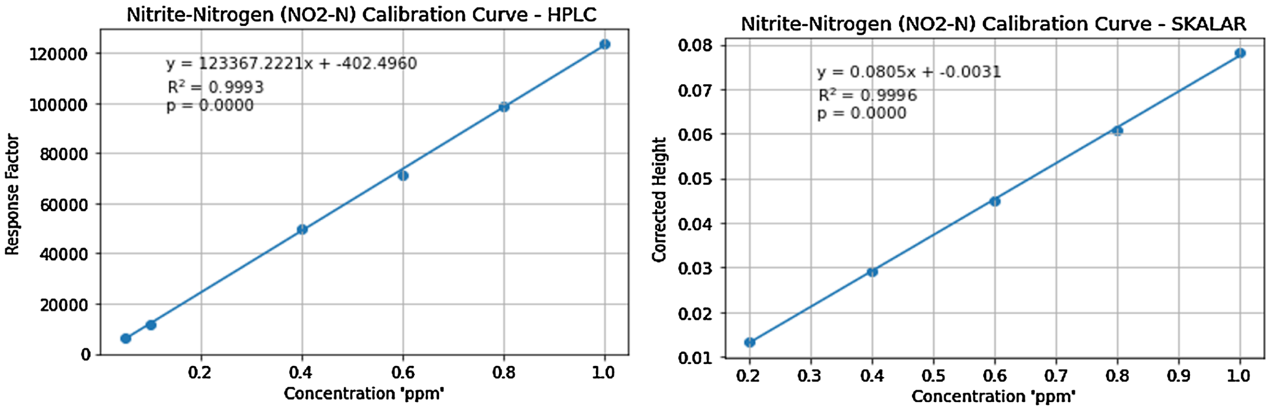
Acetonitrile HPLC grade & Methanol HPLC analytical grade were employed and the deionized water was physically treated by Milli-QSP60 System equipped with ‘Millipore’ membrane. Sulfuric Acid (10 M) used for adjusting the pH of the mobile phase.

3.3. LCS Preparation

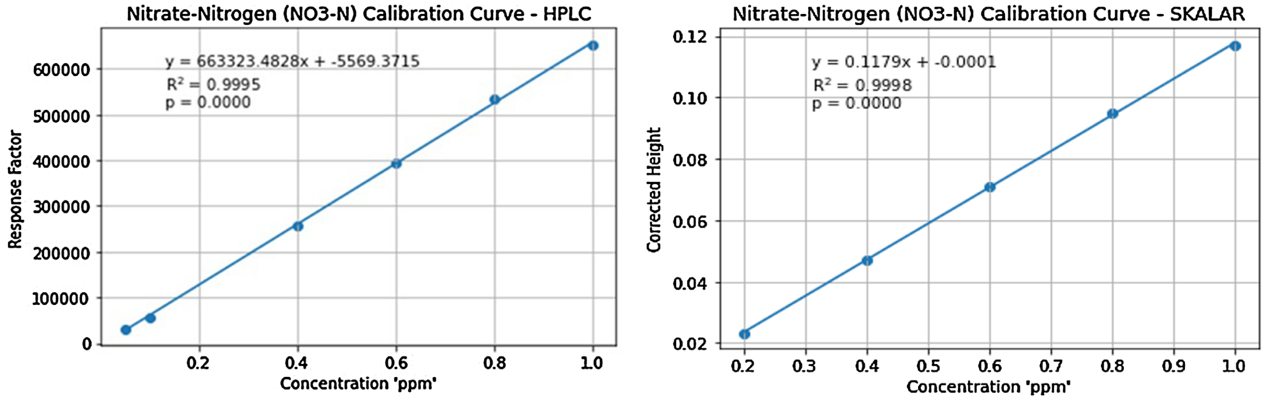
10 replicates (0.25/0.50 mg/L) of Laboratory Control Samples (LCS) for each of NO_3-N & NO_2-N were prepared from two different Certified Reference Materials (CRMs): (IC-NO_3-N-1X-1, 100 µg/mL) Nitrate as Nitrogen & (IC-NO_2-N-1X-1, 100 µg/mL) Nitrite as Nitrogen. For maintaining the integrity of the samples’ preparation, the CRM & LCS samples were stored at 4˚C inside the refrigerator prior to preparation. Calibrated Auto-pipette that is capable of accurately dispensing 0.25/0.50 mL from the CRMs into 100 mL of volumetric flask were used to prepare the LCS (0.25/0.50 mg/L) samples for both NO_3-N & NO_2-N. Deionized water from the Auto Milli-Q deionized-water dispenser was then used to dilute the pipetted-out volume of (0.25/0.50 mL) of CRM containing the analyte to the final volume of 100 mL The analysis was conducted immediately after the samples’ preparation was complete. The Qurayyah Sea Water Treatment Plant is the source from which the seawater samples for preparing spikes were obtained. **Figure 2(e)** and **Figure 2(f)** demonstrate the performance of both HPLC and SKALAR instruments by showcasing the distribution of the prepared LCS (0.25/0.5 mg/L) samples for NO_2-N and NO_3-N through boxplots.

3.4. Mobile Phase Preparation

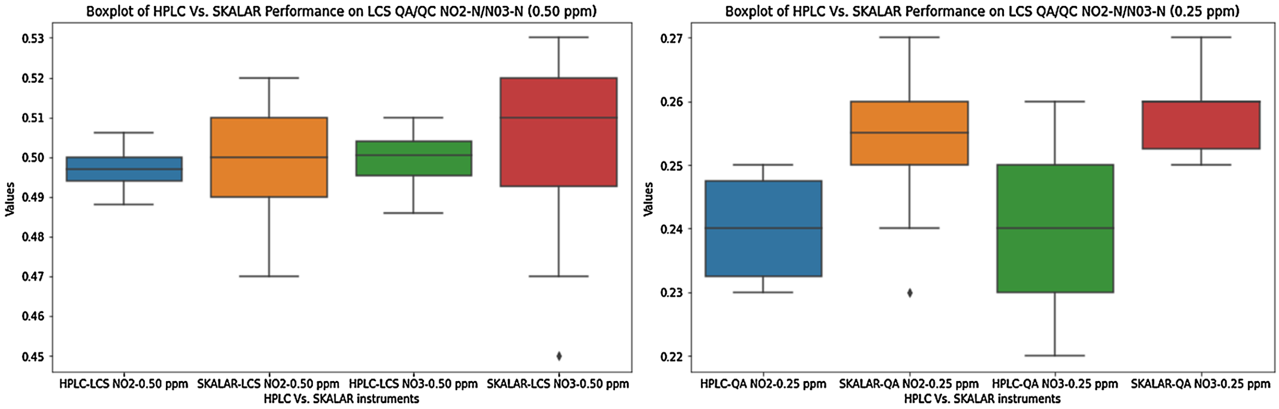
The mobile phase in HPLC plays a vital role as its parameters significantly impact the separation process, influencing retention times, resolution, and overall analytical precision by determining the interactions between analytes and the stationary phase. Optimizing the mobile phase is key to attaining optimal separation and dependable results in HPLC analyses. Different aqueous solutions of Methanol: H_2O, Acetonitrile: H_2O concentrations were tested in an attempt to find out the optimum condition of the mobile phase that can be satisfactorily employed in NO_3-N & NO_2-N analysis by the HPLC. Based on the outcomes of this study, the prepared aqueous solutions of Solvent A (Washing) of 50:50 v/v% Methanol (MeOH):H_2O, Solvent B (Seal Wash) of 10:90 v/v% MeOH:H_2O, and Solvent C (Needle Wash) of 75:25 v/v% ACN:H_2O, Solvent D (Mobile Phase) of 55:45 v/v% Acetonitrile (ACN):H_2O at the flowrate of 1.0 (mL/minute) have eventually demonstrated and contributed to the best analyte elution and separation. The deionized water of the mobile phase was collected from the Auto Milli-Q water dispenser. This deionized water was first acidified by H_2SO_4 10 M to a pH of 2.5 approximately. It is essential to maintain a low pH when assessing nitrite and nitrate concentrations in water through HPLC. The main reason of acidification in reversed-phase HPLC is typically to guarantee consistent protonation of the



(a) NO_2-N calibration curve on HPLC (b) NO_2-N calibration curve on SKALAR



(c) NO_3-N calibration curve on HPLC (d) NO_3-N calibration curve on SKALAR



(e) HPLC Vs. SKALAR for NO_2-N/NO_3-N (LCS=0.50 PPM) (f) HPLC Vs. SKALAR for NO2-N/NO3-N (QA=0.25 PPM)

Figure 2. HPLC Vs. SKALAR Nitrite Nitrate-Nitrogen statistics.

analyte, which is essential for stable retention. The capillaries utilized in HPLC contain silanol groups (Si-OH) on their inner surfaces. When the pH is sufficiently low (generally below 3.5), these silanol groups become completely protonated, resulting in a positive charge. This positively charged surface of the capillary generates a flow that opposes the standard flow observed at neutral pH. The reversed electroosmotic flow (EOF), which moves from the cathode to the anode, creates a co-EOF that aids in accelerating the movement of negatively charged nitrite and nitrate ions toward the detector. The synergistic effects of enhanced analyte mobility and reversed EOF greatly expedite the separation process, enabling ultra-rapid analysis of nitrate and nitrite in water samples (Cristiana *et al*., 2010) [7]. Then, the acidified deionized water filtered through 0.22 µm membrane. The deionized water filtrate was used to prepare the aqueous solution of Solvent A (Mobile Phase) of 55:45 v/v% ACN:H_2O.

3.5. Standards Preparation

Nitrate-Nitrogen (NO_3-N) & Nitrite-Nitrogen (NO_2-N) standards of different concentrations: 0.05, 0.10, 0.40, 0.60, 0.80, 1.0 mg/L were prepared from the CRMs for (IC-NO_3-N-1X-1, 100 µg/mL) & (IC-NO_2-N-1X-1, 100 µg/mL) respectively. The calibration curves generated displayed exceptional linearity across all tested concentration ranges. **Figure 2** as in (a) and (c) illustrate the calibration curves for NO_2-N and NO_3-N using the HPLC instrument, while **Figure 2** as in (b) and (d) depict the calibration curves for the same standard ranges on the SKALAR instrument, both showcasing impressive linearity with correlation coefficients close to 1.

3.6. Analysis by HPLC

The HPLC was first allowed to equilibrate to optimize a straight and steady baseline. The mobile phase was introduced to the C-18 column at flow rate of 1.0 mL/minute for 30 minutes, New UV lamp was installed and configured. The UV wavelength at 210 nm was chosen based on the wavelength maxima absorbance of the analyte under the UV PDA range. The injections of the standards were carried out to see if reproducible retention times and peak areas will be obtained. Retention times of both analytes were then confirmed. The peaks of the analytes were recognized by relating them to the peaks generated by the standards. The concentrations of nitrate-N and nitrite-N were calculated from the equation of the least square regression line of each standard calibration curve. The injection volume of 20 µL was selected for the best sensitivity in this experiment. Shutdown method was created to automatically rinse the HPLC system with Solvent A and shutdown the instrument after the run.

4. Results and Discussion

4.1. Implication of Switching from Skalar to HPLC

The operational consequences of transitioning from the Skalar method to the HPLC technique for analyzing NO_2-N and NO_3-N in water suggest an approximate annual cost reduction of USD 20,000. This is based on the understanding that the Skalar instrument necessitates yearly regular preventive maintenance, which involves replacing highly consumable components such as membranes, a 150 nm dialyzer, a 4-way manual switching valve, a 30 mm LR flow-cell tube, a 540 nm filter, tubes, and a glass U column macro with an active CD, including service and labor charges, as illustrated in **Figure 3** below.

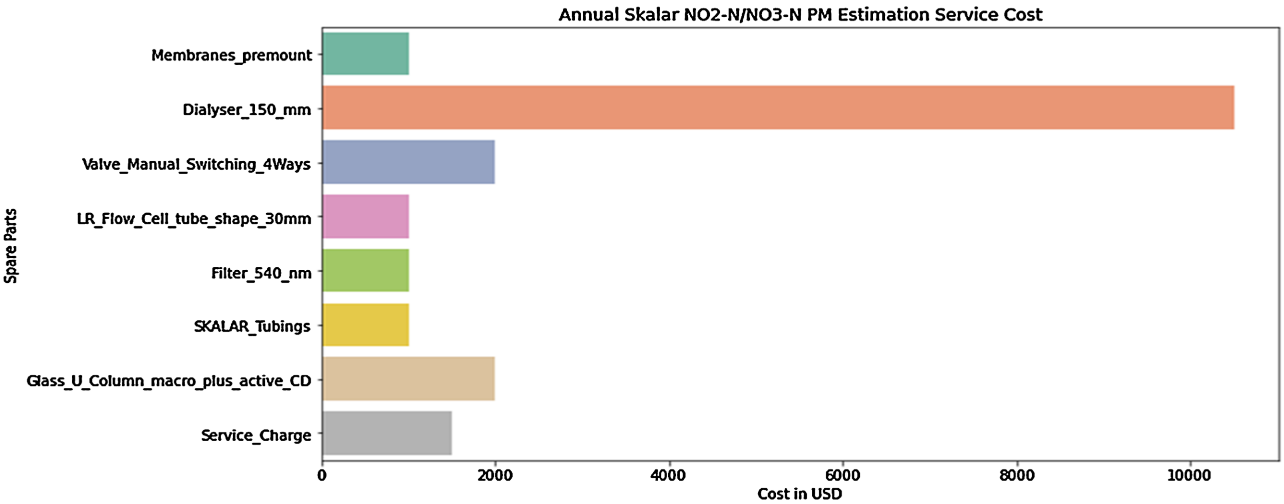


Figure 3. Estimation of the annual expenditure necessary for the preventive maintenance of the Nitrite Nitrate SKALAR Instrument to ensure its sustainability and optimal performance.

4.2. Assay by HPLC and Method Optimization

The innovative long C-18 column, along with various solvents for HPLC instrument conditioning and advanced auto-pipetting devices for preparing standards, significantly improved the separation of analytes, as well as the accuracy and sensitivity of the HPLC system. The results from the HPLC were found to be comparable to those obtained using the Cadmium reduction method (ASTM D 3867) via a continuous flow analyzer (SKALAR) for measuring trace levels of nitrate-N and nitrite-N in water. The mobile phase consisted of CH3CN and acidified H_2O in a 55:45 v/v ratio to enhance resolution and peak shape, with the acidified deionized water adjusted to a pH of 2.5. The mobile phase flow rate was set at 1.0 mL/min, and a 20 µL injection volume was utilized. Each run took only 3 minutes, with retention times for Nitrate-N and Nitrite-N recorded at 2.38 ± 0.02 and 2.63 ± 0.02, respectively. Figure 4 depicts the elution profiles of the nitrate-N and nitrite-N standards.

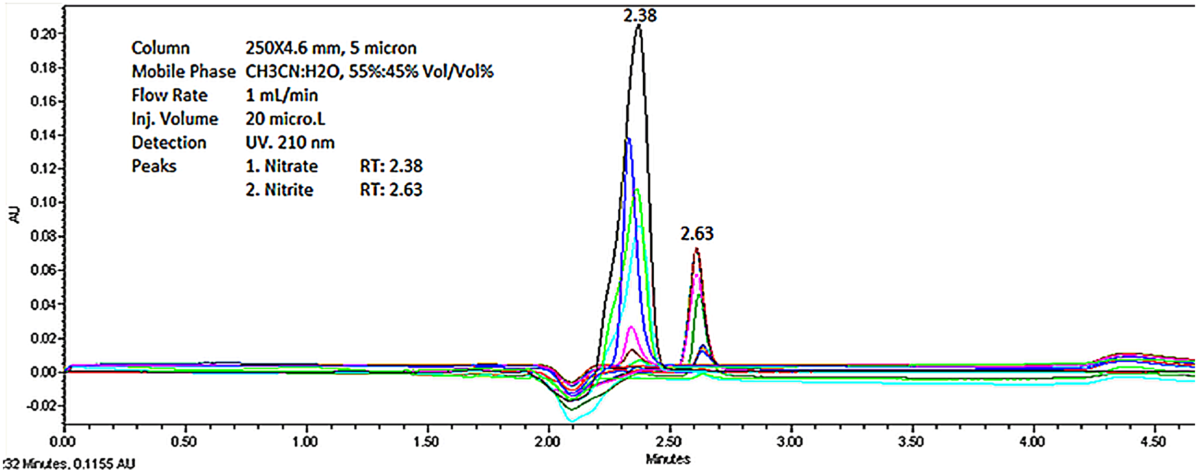


Figure 4. HPLC chromatogram of Nitrate-N and Nitrite-N standards showing analytes separation, injected concentrations: 0.05, 0.10, 0.40, 0.60, 0.80 and 1.0 mg/L. Injected volume: 20 µL.

4.3. Standard Curve Linearity

Figure 2 as in (a)-(d) demonstrate the standard curves of nitrate-n and nitrite-n over 0.05 to 1.00 mg/L concentration range. The linear regression equation for nitrate standard curve was computed as $y = 663323x - 5569.4$ with $R^2 = 0.9995$; while, nitrite standard curve calculated as $y = 123367x - 402.5$ with $R^2 = 0.9993$. The correlation coefficients for both standard curves were greater than 0.999 which was an indicative of a very good linearity.

4.4. Method Sensitivity

EPA procedure for the determination of the method detection limit (MDL) was employed to calculate the method detection limit of nitrate and nitrite. MDL was calculated by the following formula: $\text{MDLS} = t(n-1, 1-\alpha = 0.99) * S$, where

MDLS = Method detection limit based on spiked samples

$t(n-1, 1-\alpha = 0.99)$ = Student's t-value for a single-tailed 99th percentile, utilizing standard deviation with $n-1$ degrees of freedom

Ss = Sample standard deviation of the replicate spiked samples (EPA, 2016) [8]

The results showed that the method detection limit exceptionally low at 0.02 mg/L for both of both nitrate-N and nitrite-N individually. The developed HPLC method for nitrate-n and nitrite-n is very sensitive. It is capable of identifying trace levels of NO_2-N and NO_3-N as low as 0.02 mg/L.

4.5. Reproducibility and Repeatability

The reproducibility of both SKALAR and HPLC instruments was assessed for 10 QA/QC replicate samples of 0.25 mg/L NO_2-N and NO_3-N, conducted over a span of 10 days and within a 24-hour interval, as shown in Figure 2(e) and Figure 2(f). The evaluation utilized the %Relative Standard Deviation formula: $\%RSD = S/\mu*100$. Subsequently, calculations were carried out to determine the mean, sample standard deviation, and relative standard deviation, as detailed in Table 1.

Table 1. Reproducibility of QA/Q on HPLC vs. SKALAR instruments.

Analyte	QC = 0.25 of NO_2-N & NO_3-N analysis, n = 10			
	Technique	Mean (mg/L)	SDEV	%RSD
NO_2-N	HPLC	0.242	0.0042163702135	1.74
NO_3-N	HPLC	0.239	0.0031622776601	1.32
NO_2-N	SKALAR	0.253	0.0115950180873	4.58
NO_3-N	SKALAR	0.258	0.0063245553203	2.45

As shown in Table 1, HPLC exhibited a lower %Relative Standard Deviation (%RSD) of 1.32%, in contrast to the 2.45% %RSD recorded by the SKALAR instrument. These low %RSD values highlight a significant level of reproducibility.

4.6. Recovery

Table 2 shows the recoveries of nitrite-n and nitrate-n spiked with (0.25, 0.50 and 100 µg/mL) into Arabian Gulf seawater samples. The nitrate-n and nitrite-n SKALAR range was between 99.8% - 101.2% and 98.5% - 103.2% respectively. By contrast, the nitrate-n and nitrite-n HPLC range was between 96% - 99.8% and 97% - 100.3% individually. The average recoveries of nitrate-n and nitrite-n for the SKALAR was from 100.2% - 100.7%; while the average recoveries of nitrate-n and nitrite-n for the HPLC was from 98.4% - 99.1%. Given these recoveries' ranges and averages, the employed method for determining nitrate-n and nitrite-n on HPLC PDA UV Detector is accurate and comparable to the traditional automated Cadmium Reduction method by SKALAR NO_2/NO_3 instrument. Furthermore, the detection limit and recovery rate of the HPLC developed method significantly influence its practical application by determining the accuracy of substance measurements, particularly in trace amounts, and the reliability of the results. A low detection limit enables the identification and quantification of substances present in minimal quantities, which is crucial in fields such as environmental monitoring. For instance, it helps in identifying trace levels of pollutants; whereas a high recovery rate ensures that the measured quantity of the substance accurately reflects the actual amount in the sample, thereby minimizing errors.

Table 2. Recoveries of NO_2-N and NO_3-N returns from HPLC and SKALAR instruments.

Analyte	Standards of NO_2-N & NO_3-N analysis				
	Technique	0.25 mg/L	0.50 mg/L	1.0 mg/L	Average (%)
NO_2-N	HPLC	97.0%	100.0%	100.3%	99.1%
NO_3-N	HPLC	96.0%	99.4%	99.8%	98.4%
NO_2-N	SKALAR	103.3%	101.6%	98.5%	100.2%
NO_3-N	SKALAR	101.2%	101.0%	99.8%	100.7%

5. Conclusion

The developed technique for analyzing nitrate-n and nitrite-n employs the HPLC Water 2695, paired with a 2998 Photodiode Array UV Detector, utilizing a non-polar Luna Column-18 measuring 5 µm × 250 µm. The polar mobile phase consists of CAN and H_2O (acidified to pH 2.5) in a 55% to 45% ratio, operating at 40˚C with a flow rate of 1 mL/min and an injection volume of 20 µL, at a wavelength of 210 nm. This method is user-friendly, rapid, precise, and highly sensitive. Unlike the SKALAR instrument, it conserves water resources and eliminates the need for complex sample preparations or the production of hazardous waste, which is often associated with the cadmium reduction method used in SKALAR method. Additionally, the HPLC method requires significantly less sample volume (20 µL) and deionized water. The statistical analysis revealed that the methodologies of the two instruments are largely comparable and precise. However, the cadmium waste generated by the SKALAR instrument poses a significant environmental risk if not handled correctly, potentially leading to severe water pollution. Maintaining the SKALAR instrument demands extensive periodic upkeep and frequent replacement of high-cost consumable parts to ensure its optimal performance. In contrast, the HPLC system does not generate hazardous waste that could adversely affect water resources. Consequently, HPLC proves to be more cost-effective, reliable, short turnaround time, capable of higher throughput, requires less maintenance, conserves water, is safer, and does not produce cadmium-contaminated waste. Unlike the SKALAR instrument, HPLC is regarded as more environmentally friendly.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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