



ISSN Print: 2394-7500
 ISSN Online: 2394-5869
 Impact Factor: 8.4
 IJAR 2023; 9(6): 274-282
www.allresearchjournal.com
 Received: 11-03-2023
 Accepted: 13-05-2023

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Water testing in marine aquaculture: Method optimization and validation for ammonium quantification

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Abstract

Ammonia has wide range of applications globally, with maximum use as a fertilizer in agriculture and aquaculture. However, concentration of ammonia above a certain threshold becomes toxic to aquatic fauna. Therefore, the right and accurate quantification of ammonium is important for checking water quality. Colorimetric, high pressure liquid chromatography and ion chromatography are widely used methods for ammonium estimation in water and wastewater samples. Because of cost effectiveness, the colorimetric method is most preferred among all these. However, the colorimetric method is not suitable for marine samples due to the presence of interfering agents such as salts, ions, metals, and biological contaminations. In this study, salicylate method was optimized and validated for ammonium estimation in standard and test samples (marine). In the study, 653 nm wavelength was selected to measure the absorbance and the method was found linear till 70 ppm ammonia concentration. Using this method 100% ammonium was accurately estimated from standard and test samples. The repeatability study conducted with 10 replicates showed that, the method was more precise and less prone to errors. The ruggedness study conducted with different analysts and days showed that the results were more consistent with less variations. Also, the variation in wavelength (± 10 nm), reagent's volume ($\pm 100 \mu\text{l}$) and incubation time (± 10 min) did not affect the results significantly and method was found more robust. The validated method has a limit of detection of 0.5 ppm ammonium; hence it can be applied to measure such lower concentrations in marine water. Overall, study clearly shows that the developed salicylic acid method is more specific, linear, accurate, repeatable, rugged, robust and more sensitive for ammonium estimation in marine aquaculture samples.

Keywords: Ammonium, marine water, salicylic acid, UV-VIS spectrophotometer

Introduction

Ammonia is a colorless gas and has characteristic pungent smell. It's lighter than air and easily liquefied due to the strong hydrogen bonding between molecules. The liquid boils at -33.3°C and freezes to white crystals at -77.7°C . Ammonia is a precursor to most nitrogen-containing compounds and virtually all synthetic nitrogen compounds are derived directly or indirectly from ammonia (Holleman, A *et al* 2001) [6].

Ammonia has wide range of applications worldwide. It is used as cooling agent, electrolyte in dry cells, anti-coagulant in latex, fertilizer, synthetic fibers and explosives production. Among all these applications, maximum consumption of ammonia was reported as a fertilizer which is around 88% and could be used in various forms such as salts, solutions or anhydrous (Tam NFY, *et al.* 1990) [14]. The ammonia nitrogen in aquaculture systems primarily comes from fertilizers as well. Ammonia is the next most important factor after low dissolved oxygen concentration, limiting the number of fish that can be produced in aquaculture system. High concentrations of ammonia, however, is toxic to aquatic animals at above 0.2 mg/L (Ogbonna *et al* 2010) [8]. Poor growth and feed conversion rates, reduced fecundity and fertility, and susceptibility to bacterial infections and disease have been reported in fish at higher concentrations of ammonia. Elevated ammonia nitrogen in water can cause gill damage, oxygen-carrying capacity reduction in the bloodstream, lack and depletion of adenosine triphosphate (ATP) in the brain, and liver and kidney malfunction (Zhou, Li & Boyd, Claude, 2015) [16].

Moreover, ammonia along with phosphorus when discharged to the environment contributes to the eutrophication of water bodies. Hence, the concentration of ammonia in fishpond/aquaculture ponds should be closely monitored to avoid both chronic and acute toxic effects. Therefore, accurate and precise estimation of residual ammonium is required to keep its toxicity under check (Crofts AR, *et al.* 1966)^[3].

Various analytical methods are available for measurement of ammonia/ammonium from different types of samples. Spectrophotometric Nessler's reagent (HJ 535-2009) method (HJ 536-2009) is recommended for the determination of ammonium in liquid samples (H Wang *et al.* 2013)^[4]. Traditionally, Kjeldahl distillation methods are used to estimate ammonium levels from biological tissues. In Kjeldahl distillation, ammonia is distilled and subsequently trapped in acid and estimated by titrimetric or colorimetric process. In this method there is a chance of overestimation due to the cleavage of protein amino groups and the formation of ammonia by deamination reactions (Parris and Foglia 1983)^[11]. Ammonia is also estimated by using ammonia-selective electrodes, enzymatic assays, and chromatographic techniques. However, some discrepancies were observed in terms of results between electrodes and enzymatic procedures. The selective electrodes respond to both ammonia and volatile amines, whereas enzymatic procedure is more specific to ammonium (Parris and Foglia 1983)^[11]. Chromatographic separation techniques are more popular because of their specificity (Huizenga *et al.* 1994; Parris 1984)^[7, 17]. Salicylic acid method is a very cost-effective colorimetric method used for ammonium estimation from water samples. This is a variant of indophenol method; in this method the phenol was replaced with sodium salicylate to avoid the emission of toxic compounds such as o-chlorophenol. Salicylic acid method has been proven more precise than indophenol blue as per the available literatures (P Le *et al.* 2012)^[10]. In this study, salicylic acid method was validated, to verify its specificity, accuracy, precision, robustness, sensitivity and suitability for estimation of ammonia from marine water samples.

Materials and Methods

Materials

Standard stock solution – 1000 ppm NH₄⁺, filtered marine water and Milli-Q water was used as blank as well as diluent. UV-VIS Spectrophotometer (UV-1800). *Picochlorum* sp. grown in marine water containing ammonium. All reagents and chemicals of laboratory reagent grade were used in the study. All glass-wares were used of class A and were washed with 1 N hydrochloric acid (HCl), washing solution, tap water and dried at 60 °C for 4-5 hrs.

Inoculum Developed for Validation Study

The *Picochlorum* sp. was obtained from Reliance Industries Ltd. germplasm repository. The *Picochlorum* sp. was maintained on urea phosphoric acid (UPA) agar slants. The inoculum was developed in UPA medium under standard controlled conditions in Percival chamber with temperature of 27±2 °C, pH 7.0-7.5, 4% salinity, 2% CO₂ (during light period) and 200 μE m⁻² s⁻¹ of light intensity at 12:12 light/dark cycle. The culture was grown till the stationary phase and whole culture was used for validation study after

centrifugation, by spiking with various known concentrations of ammonium.

Solution Preparation

Reagent-1

(Sodium Salicylate and Sodium Nitroprusside Solution)

Reagent 1 solution was prepared by dissolving sequentially 10 g/L of NaOH, 200 g/L sodium salicylate, 1.6 g/L of sodium nitroprusside in 1000 mL of Milli-Q water.

Reagent-2 (Hypochlorite and NaOH Solution):

The reagent 2 solution was prepared by dissolving sequentially 5 g/L of NaOH and 75 mL/L (4% w/v) of sodium hypochlorite in 1000 mL of Milli-Q water.

Standard and Sample Solutions

Thousand ppm of ammonium stock solutions A and B were prepared by dissolving 3.13 gm of previously dried ammonium chloride in Milli-Q water and marine water samples respectively. Sub stocks of 70 ppm were prepared from the above stocks. The sub stock prepared in milli-Q water was considered as standard and the sub stock prepared in marine water sample was named as sample solution. The standard solution (prepared in Milli-Q water) and sample solution (prepared in marine water) were directly used for validation study, and respective water samples were used for sample dilutions.

Instrumental Analysis

UV-Vis Spectrophotometer (UV-1800 by Shimadzu Scientific Instruments Inc.) was used for method validation. This instrument works on Beer-Lambert law, according to this the absorbance of a solution is directly proportional to the concentration of the absorbing material in the solution. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorbent in a solution.

Salicylic Acid Method Principle and Chemicals Reaction

Compounds of ammonium initially reacts with hypochlorite to form monochloramine, which then reacts with salicylate to form 5-aminosalicylate which gets oxidized in the presence of a catalyst, nitroprusside or nitro-ferricyanide (Fe(CN)₅NO₂⁻), forming indo-salicylate, a blue-coloured compound. The blue colour is masked by the yellow colour (due to excess nitroprusside) causing a green-coloured solution. The intensity of the colour is directly proportional to the ammonia concentration in the sample.

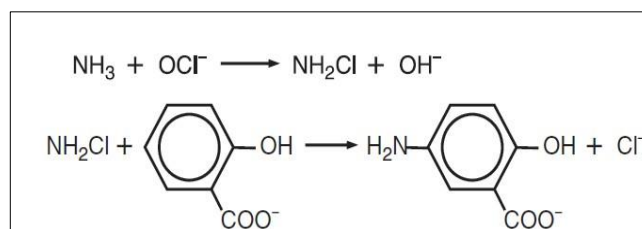


Fig 1: Chemical reaction for ammonium estimation

Ammonia Estimation Protocol

To the previously washed 10 ml of dried glass tubes 1750 μL of ammonia reagent -1 followed by 100 μL blank/standard/samples and 1750 μL ammonia reagent-2 were added. The reagent and sample were mixed thoroughly and incubated at room temperature (RT) for 30 minutes. The

absorbance was measured at 653 nm against respective blanks and concentration of the ammonia was estimated using slope value as per below formula. Slope was generated during linearity study from 0 to 70 ppm ammonium concentration.

Ammonium Concentration (ppm) = Absorbance / Slope Value

Analytical Method Validation

Validation of any analytical method is a process which proves that the specific method can provide precise and accurate analytical results. Since, only quality data would be acceptable for the intended use. The salicylic acid method of ammonia estimation by UV-VIS spectrophotometer was validated as per the international council for harmonization (ICH) guideline of analytical method validation (ICH Guideline 1996) [18].

Specificity

Standard and sample solutions were used for specificity study after dilution to 5, 10, 20, 30, 40, 50 and 60 ppm ammonia concentrations using Milli-Q water and filtered marine water as diluent respectively. The spectrum was scanned between 500 nm to 800 nm using UV-1800 for all the concentrations prepared from standard and sample solutions. The mean of maximum absorbance for each concentration was recorded for all solutions. The absorbance maxima for sample and standards were calculated from the spectra taken from 500-800 nm. The percent relative standard deviation (% RSD) of 5% was considered for specific absorbance on 653 nm for all dilutions of standard and sample. Similarly, 1% RSD was considered for mean absorbance maxima for all dilutions of standard and sample.

Linearity

It is calculated in accordance to established mathematical relationship among the test results obtained by the analysis of ammonium at varying concentrations. Six different concentrations such as 10, 20, 30, 40, 50 and 60 ppm were prepared in two different sets from standard and sample sub stock solutions of 70 ppm. In a set of standards, Milli-Q water and for a set of samples, marine water was used as diluent. Linearity curve was plotted between ammonium concentration and observed absorbance; correlation coefficient was also calculated for the same at each level. Acceptance criteria for correlation coefficient of the regression line (R2 value) was considered at minimum 0.98.

Accuracy

The accuracy study was carried out to evaluate the recovery of salicylic acid method. Standard and sample solution of 30 ppm were prepared for this study. Three different concentrations of ammonium 35, 45 and 50 ppm were prepared by spiking known amount ammonium stock solution in standard and sample solutions. All concentrations were analysed in triplicates and percent recovery of ammonium was calculated. Acceptance criteria for percent recovery was considered at $100 \pm 5\%$ as per the ICH guideline.

Precision

Previously prepared standards and samples were used for various studies of precision.

- **Repeatability:** Ammonium concentration were analyzed in replications of 10 for standard and sample solutions having 70 ppm concentration, to check the repeatability of the salicylic acid method. Percent RSD for absorbance and ammonium concentration were calculated, and acceptance criteria were considered at maximum of 1% and 2% respectively.
- **Ruggedness:** Standard and sample solution having 70 ppm ammonium concentration were used for ruggedness analysis. Two studies were performed to understand the ruggedness of this analytical method. During the first study both standard and sample were analysed in replications of 5 at two different days while in second study same standard and sample were analysed in replications of 5 by two different analysts. The percent RSD for ammonium concentration of standard and sample were calculated and acceptance criteria for percent RSD was considered at maximum 2.0% for ammonium content.
- **Robustness:** Standard and test sample solution of 70 ppm were used for robustness analysis. This analysis of salicylic acid method was performed by varying three operational parameters, which were wavelength, reagent's volume and incubation time. All these studies were performed separately in replications of 5, by varying one parameter at a time to understand the robustness of this analytical method. During the first study wavelength was deviated by ± 10 nm, in the second study reagent-1 and 2 were deviated (1750 ± 100 μ l) one by one separately and finally during the third study incubation time was deviated 30 ± 10 minutes. Percent RSDs were calculated for ammonia concentration and acceptance criteria of maximum 2% was considered for ammonium content.

Sensitivity

Two solutions of 10 ppm ammonium concentration were prepared from standard and sample solution having 70 ppm ammonia concentration in the respective diluent. Seven various concentrations such as 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm were prepared from 10 ppm respective stocks. The absorbance was estimated in duplicate for all dilutions of ammonium using salicylic acid method.

Sensitivity was measured by analyzing limit of detection and limit of quantification using Micro- soft excel.

Limit of Detection and Limit of Quantification: In analytical science limit of detection (LOD), is the lowest quantity of any substance which can be detected with a stated confidence. Limit of quantification (LOQ) is the lowest analyze concentration that can be quantified with a stated accuracy and precision. The linearity curves were plotted between concentration and absorbance for standard and sample solutions, slope values were also calculated separately from linearity curves. Standard error (SE) & standard deviation (SD) values were calculated from regression statics with the help of Microsoft excel. LOD and LOQ values were calculated as per below mentioned formula using slope and SD.

$LOD = 3.3 * (SD / Slope)$

Where 3.3 is the constant (ICH Q2 R1)

LOQ= 10*(SD/Slope)
 Where 10 is the constant (ICH Q2 R1)

Results and Discussion

Specificity

According to the recorded UV spectrum, the maximum absorbance was observed at wavelength of 653 nm for all various concentrations of standard as well as sample (Fig.

2A and 2B). Absorbance were found linear to the concentration and comparable between standard and sample solutions. As per the observed data, the mean percent RSD of 2.52% and 2.99% were observed for the measured maximum absorbance of standard and solution respectively. These results indicate that measurements clearly meet the acceptance criteria of 5% and confirms the specificity of the method.

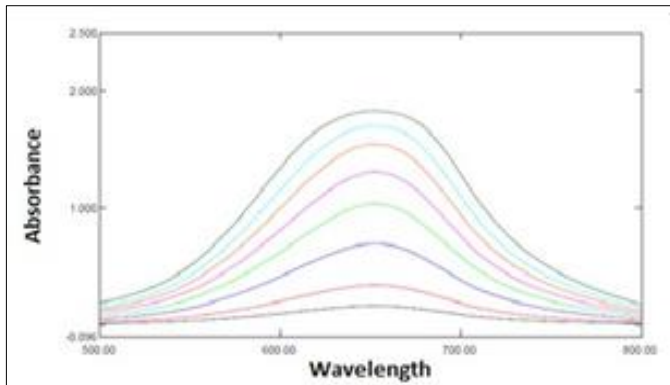


Fig 2A

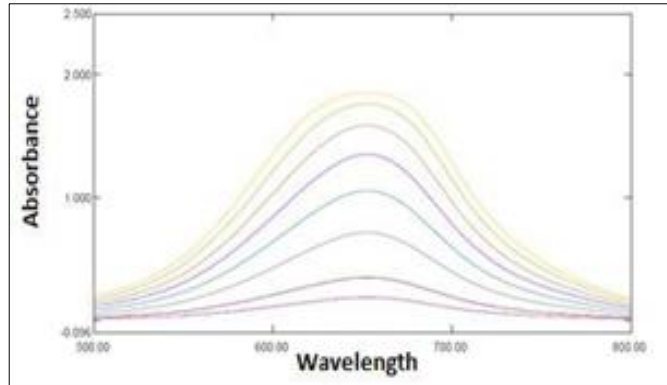


Fig 2B

Fig 2: UV Spectrum for various concentrations prepared standard solution (2A) and sample (2B)

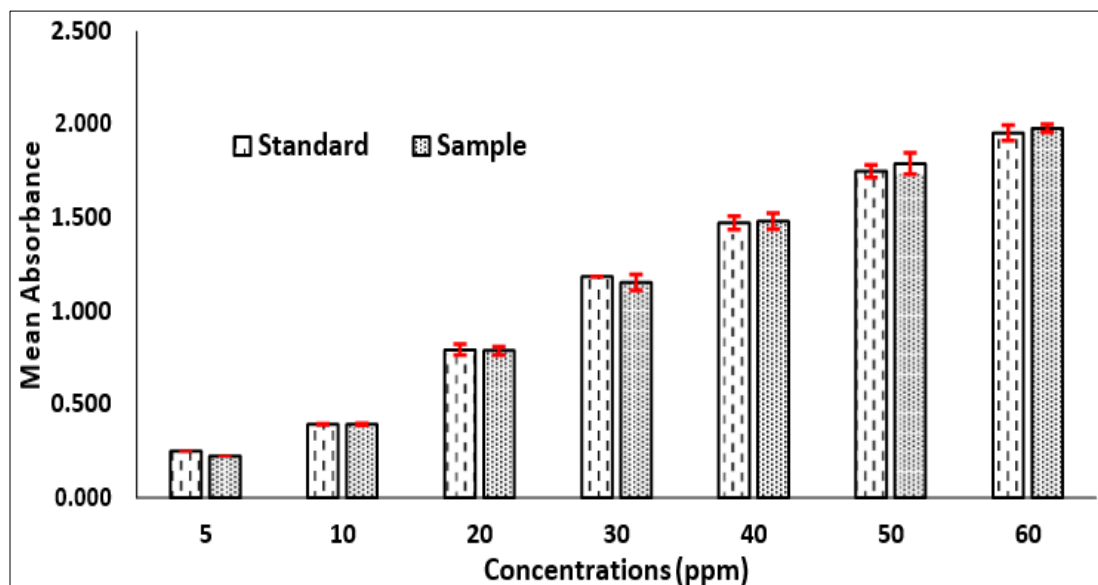


Fig 3: Mean absorbance values for standard and sample solutions

Linearity

The linearity curve of absorbance and ammonium content from standard and sample have shown correlation coefficient of (R2 value) 0.9965 & 0.9971 respectively. These curves were found statistically significant and also met the acceptance criteria (> 0.98) of ICH guideline. The slopes value of 0.0438 and 0.0421 were determined for standard and samples respectively (Fig 4 & 5). Fresh slope values were calculated on daily basis and same used for calculation of ammonium concentration during multiple studies of validation. Because of two different media (Milli-Q water and marine water), minor differences in slope values were observed. The slope of Milli-Q water medium was found ~3.9% higher than marine water medium, however it was found statistically insignificant. According

to the results and linearity curves, this analytical method is found suitable to analyse ammonium concentration till 70 ppm in both media (Milli-Q water and marine water) and above which it was not found linear. Since this is a colorimetric technique and method is not linear above 70 ppm, hence pre-dilution is necessary to estimate ammonium from the sample which contain > 70 ppm. M. Arvand *et al*; (2020) [9] validated analytical method of ammonium estimation by ion chromatography and during linearity study, regression line was obtained with a correlation coefficient of 0.9996 for ranging from 0.008-0.3 mM ammonium ion. Reported R2 value by Arvand *et al*; was slightly higher as compared salicylic acid method however, both methods have met the acceptance criteria for correlation coefficient.

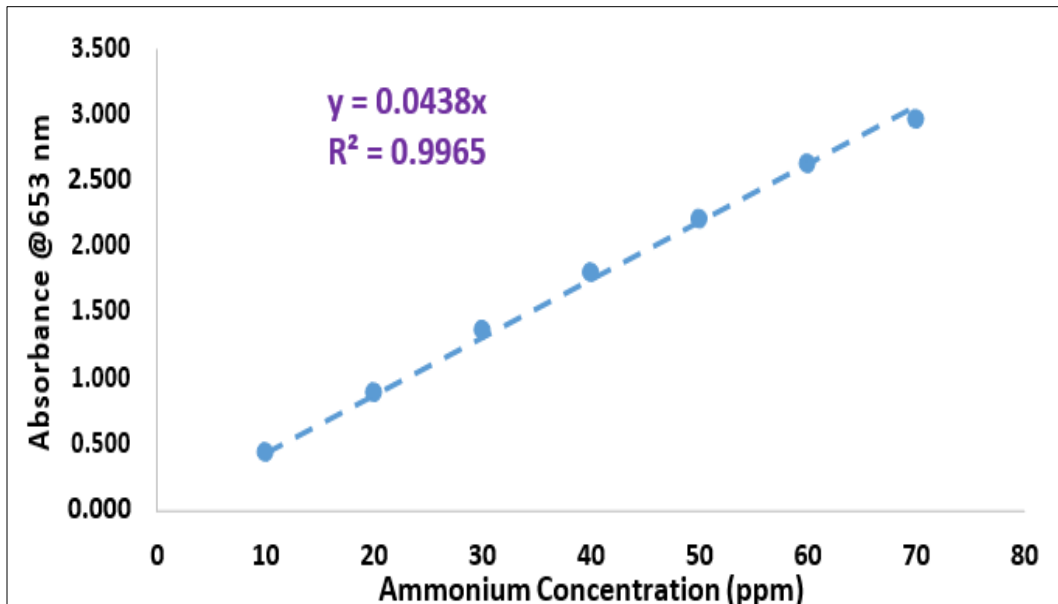


Fig 4: Linearity curve – standard

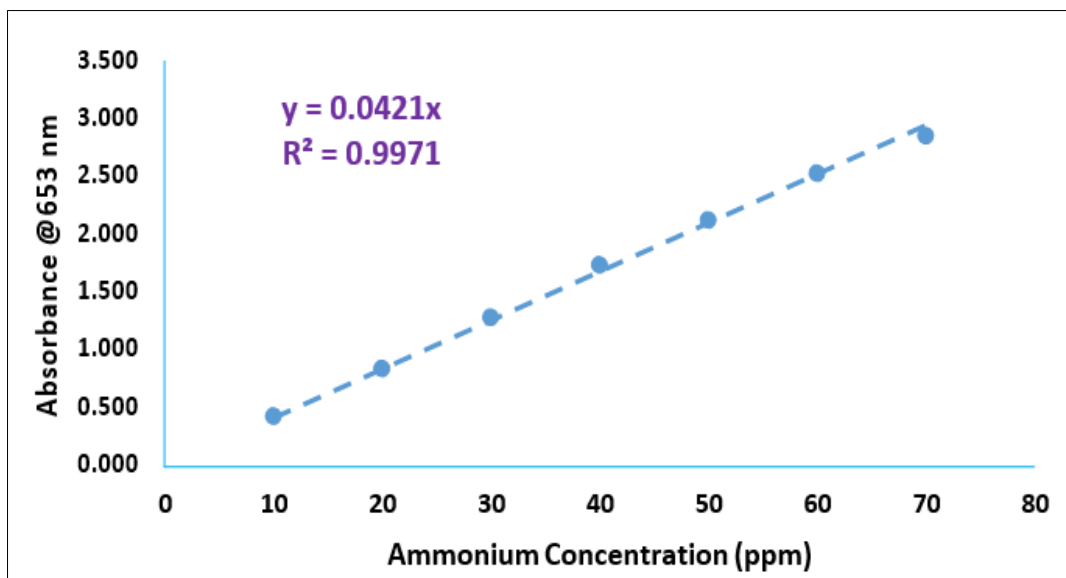


Fig 5: Linearity curve–sample

Accuracy

In recovery study with standard solutions of 35, 45 and 50 ppm ammonium, 99.2%, 101.1%, and 101.3% recoveries were observed respectively with a mean recovery of 100.5% (Table 1). Whereas, in case of sample 102.4%, 103.7%, and 101.3% recoveries were observed respectively, with a mean recovery of 102.5% (Table 2). The results of accuracy have demonstrated that, this analytical method of ammonium estimation meet the acceptance criteria of $100 \pm 5\%$ for mean percentage recoveries and results were found satisfactory. This analytical method was found suitable for the accurate quantification of ammonium from fresh water as well as marine water samples. Detailed accuracy/recovery results are summarized in Table-1 and 2. It was observed

that in sample solutions 2.0% higher recovery was observed as compared to standard solution. The higher recovery in sample solution may be due to dissolved ammonium ions in marine water. However, estimation from both types of samples have meet the acceptance criteria and proved that salicylic acid method can recover more than 95% concentration of ammonium. According to the accuracy study by M. Arvand *et al*; (2020)^[9], the individual percentage recovery of ammonium varied from 95.9 to 98.1% for all three levels, and the mean percentage recovery of 96.9% was observed for ion chromatography method. Salicylic acid method has shown slightly higher recovery as compared to ion chromatography method.

Table 1: Summarized results of accuracy/recovery-for standard solution

Sr. No.	Mean Theoretical Concentration (ppm)	Mean Estimated Concentration (ppm)	% RSD	% Recovery	Acceptance Criteria
1	34.9	34.6	0.32	99.2	100± 5%
2	43.7	44.2	1.57	101.1	
3	50.3	51.0	2.56	101.3	

Table 2: Summarized results of accuracy/recovery-sample solution

Sr. No.	Mean theoretical Concentration (ppm)	Mean Estimated Concentration (ppm)	% SD	% Recovery	Acceptance Criteria
1	36.0	36.9	0.38	102.4	
2	44.9	46.6	0.19	103.7	100± 5%
3	51.6	52.3	0.61	101.3	

Precision

Repeatability: In case of standard sample, mean absorbance of 2.809 and mean ammonium concentration of 68.7 ppm were reported. Similarly, in case of sample, mean absorbance of 2.802 and mean ammonium concentration of 68.5 ppm were reported. The RSDs of 0.82% and 0.52%

were observed for standard and sample respectively (Table-3). All the values of RSDs were within the acceptance criteria of 1% and 2% for absorbance and ammonium concentration respectively. This study proves that the repeatability of salicylic method is satisfactory.

Table 3: Summarized results of repeatability

S. No.	Standard Solution		Sample Solution	
	Absorbance	NH4 ⁺ (ppm)	Absorbance	NH4 ⁺ (ppm)
Replicate 1	2.788	68.2	2.803	68.5
Replicate 2	2.819	68.9	2.789	68.2
Replicate 3	2.79	68.2	2.801	68.5
Replicate 4	2.808	68.7	2.806	68.6
Replicate 5	2.793	68.3	2.813	68.8
Replicate 6	2.834	69.3	2.790	68.2
Replicate 7	2.853	69.8	2.776	67.9
Replicate 8	2.823	69.0	2.807	68.6
Replicate 9	2.785	68.1	2.830	69.2
Replicate 10	2.793	68.3	2.802	68.5
Mean	2.809	68.7	2.802	68.5
STDEV	0.02	0.56	0.015	0.36
% RSD	0.82	0.82	0.52	0.52

Ruggedness

Mean ammonium concentration of 73.7 and 71.5 ppm was observed for the standard analyzed on day 1 and day 2 respectively. Similarly, for the samples, the mean ammonium concentration of 72.9 and 70.8 ppm was observed on day 1 and day 2. During the study by different analyst, mean ammonium concentration of 71.4 and 70.7 ppm was observed for standards. In case of sample the mean ammonium concentration of 70.5 and 70.4 ppm was observed (Fig 6). During the reproducibility analysis with standards, the RSDs of 0.86%, 0.75% were observed for

ammonium measured on day 1 and day 2 respectively. Whereas, in case of different analysts the RSD of 1.06% (analyst 1) and 1.50% (analyst 2) were observed for ammonium concentration. In case of sample, RSDs 0.29% on day 1, 1.0% on day 2, 0.61% by analyst 1 and 1.56% by analyst 2 were observed (Fig 6). As per the observed data all values of ammonium concentrations were found statistically similar and percent RSDs were also within the acceptance criteria of 2%. This study proves that there is no impact of different days and analysts on estimation of ammonia by salicylic acid method.

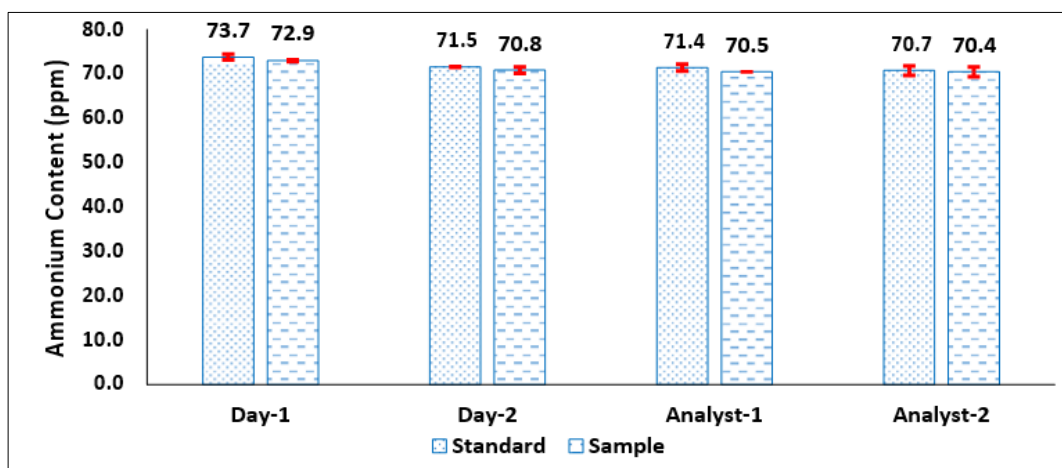


Fig 6: Mean ammonium content (ppm) analysis while evaluating the ruggedness of the method

Robustness at Different Wavelength

The ammonium concentration was calculated by measuring the absorbance at 643,653 and 663 nm were found to be 71.3, 73.7 and 70.4 ppm respectively in standard. Whereas, in case of sample, it was 72.2, 73.0 and 72.1 ppm at the respective wavelengths. In case of standard and sample, the

RSDs of < 2% was observed at various wavelengths (Fig 7). The results indicate that the wavelength is not significantly affecting the estimation of ammonium concentration and the broad range of wavelength from 643 to 663 could be used for measuring the ammonium.

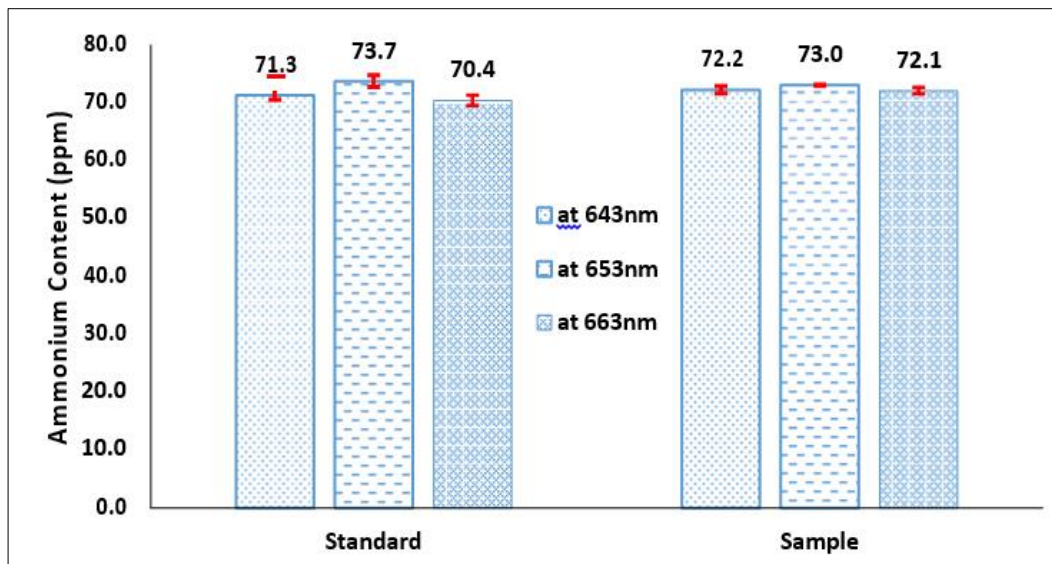


Fig 7: Mean ammonium content (ppm) measured at various wavelengths for standard and sample

Robustness at Different Volume of Reagents

The ammonium concentration measured from standard using the varied reagent 1, were found to be 69.7, 72.4 ppm at reagent 1 ±100 µl (1.85+1.75 mL and 1.65+1.75 mL) respectively as compared to 70.5 ppm at standard reagent combination (1.75+1.75 mL). Whereas in case of sample the ammonium concentrations were found to be 72.1 and 72 ppm at reagent 1 ±100 µl respectively as compared to 70.3 ppm at standard reagent combination. The RDS of < 2% was observed for standard and sample with reagent 1±100 µl (Table 4).

In case of reagent 2±100 µl (1.75+1.85 mL and 1.75+1.65 mL) the ammonium concentration measured from standard using the varied reagent 2 were found to be 70.1, 73.8 ppm respectively as compared to 70.5 ppm at standard reagent combination (1.75+1.75 mL). Whereas, in case of sample the ammonium concentration of 69.8 and 73.1 ppm was measured at reagent 2±100 µl respectively as compared to 70.3 ppm at standard reagent combination. The RSDs of < 2.5% was observed for standard and sample with reagent 2±100 µl (Table 5).

Table 4: Robustness data by deviating volume of reagent-1

Sr. No.	Ammonium Content (ppm)-Standard			Ammonium Content (ppm) -Sample		
	1.85+1.75 mL	1.75+1.75 mL	1.65+1.75 mL	1.85+1.75 mL	1.75+1.75 mL	1.65+1.75 mL
1	70.5	70.4	72.0	72.5	69.6	72.7
2	69.5	70.5	72.0	72.0	70.9	71.5
3	69.1	70.4	72.4	71.5	70.6	73.0
4	70.0	70.6	73.5	71.4	70.9	71.3
5	69.2	70.3	72.3	73.0	69.4	71.5
Mean	69.67	70.5	72.5	72.1	70.3	72.0
SD	0.60	0.11	0.63	0.68	0.70	0.78
% RSD	0.86	0.15	0.86	0.95	1.00	1.08

Table 5: Robustness data by deviating volume of reagent-2

Sr. No.	Ammonium Content (ppm)-Standard			Ammonium Content (ppm) -Sample		
	1.75+1.85 mL	1.75+1.75 mL	1.75+1.65 mL	1.75+1.85 mL	1.75+1.75 mL	1.75+1.65 mL
1	70.1	70.4	74.3	69.9	69.6	75.0
2	70.1	70.5	74.1	71.8	70.9	74.3
3	70.4	70.4	74.3	68.9	70.6	71.3
4	70.0	70.6	71.5	69.6	70.9	73.6
5	69.8	70.3	74.6	68.9	69.4	71.2
Mean	70.1	70.5	73.8	69.8	70.3	73.1
SD	0.22	0.10	1.26	1.20	0.70	1.78
% RSD	0.32	0.15	1.71	1.72	1.00	2.43

Robustness at Different Incubation Time

Estimation of ammonium from standard during varied incubation time of 30 ± 10 minutes (40 and 20 minutes), the ammonium concentrations of 71.5 and 70.9 ppm were measured respectively as compared to 70.2 ppm at standard incubation time of 30 minutes. While in case of sample the ammonia concentrations of 69.0 ppm were observed at 30 ± 10 minutes incubation time and 69.9 ppm of ammonia was

reported for standard incubation time (Fig.8). The RSDs value of < 1% was observed during the varied incubation time of ammonia estimation from standard and sample. It was found from the obtained results that; the ammonium estimation was not affected by the incubation time of 30 ± 10 minutes. As the results were found consistent with < 5% variation, the method was complying for robustness analysis.

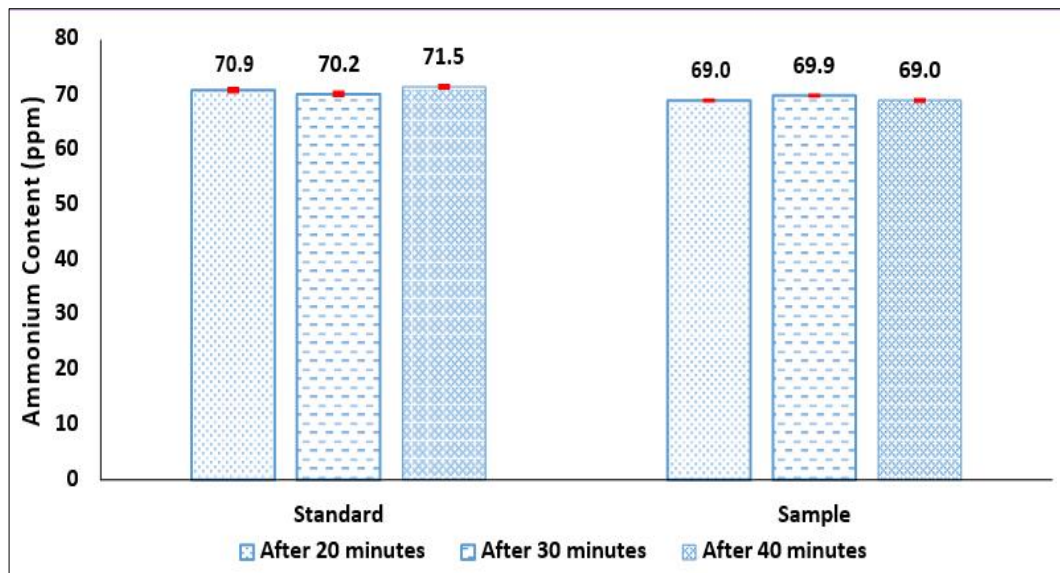


Fig 8: Mean ammonium content (ppm) at different incubation time

Sensitivity:

Analytical sensitivity, LOD and LOQ are used to describe the smallest concentration of a compound for detection and quantification by an analytical procedure.

The linearity curve developed for standard shown R² value of 0.998 and slope of 0.0495 (Fig.9).

Based on this, the method had shown LOD of 0.50 ppm and LOQ of 1.50 ppm for standard. In case of sample solution, R² value of 0.999 and slope of 0.0545 (Fig. 10) were observed. The LOD and LOQ values calculated using this slope was found to be 0.41 ppm and 1.25 ppm respectively. This study proves that, this analytical method is found suitable to detect and quantify ammonium concentration as low as 0.50 and 1.50 ppm respectively in both standard and sample solution.

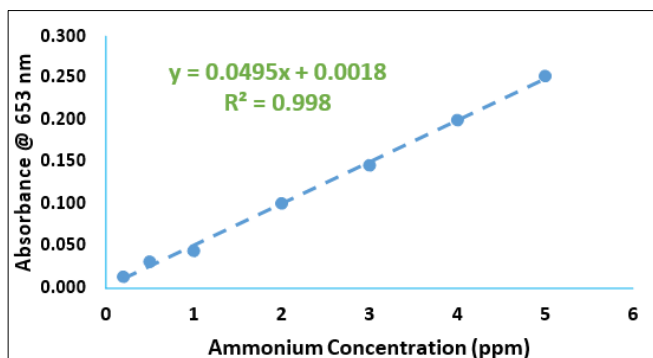


Fig 9: Linearity curve for sensitivity analysis –standard

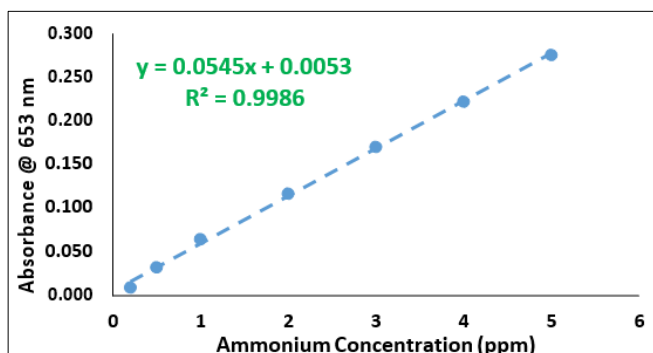


Fig 10: Linearity curve for sensitivity analysis –sample

Conclusion

According to the data of validation and specificity study the absorbance maxima of ammonium was found 653nm. The variation in wavelength, reagent, and incubation time by 5-10% was not found to affect the accuracy of the method. From precision study, the method was found repeatable, rugged, and robust and the percent RSD were found within the acceptance criteria. Statistically significant R² values were observed from the linearity curves of standard and samples. The method was found linear till 70 ppm of ammonium and can be used to detect 0.5 ppm ammonium (LOD) and quantify 1.5 ppm (LOQ) from both the medium. This method has m, *et al.* The criteria of validation, as per the ICH guidelines. This method found suitable for accurately estimation of ammonium from any biological materials/ water samples without various interferences such as ions, salts, metals and biological contaminations.

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