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UV-Vis Spectrophotometer as an Alternative Technique for the Determination of Hydroquinone in Vinyl Acetate Monomer

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Abstract: As an appropriate quantity of hydroquinone (HQ) is essential to safeguard inhibition characteristics by eliminating the risk of self-polymerization of vinyl acetate monomer (VAM), the determination of the HQ content in VAM is very crucial to ensure the stability of VAM during storage and transportation as well as to achieve the possibility of a proper polymerization reaction. In this study, a simple, cheap, time-saving, and easy method has been developed by which the HQ content in VAM can be measured quickly based on the measurement of UV-Vis absorbance of the HQ content at 293 nm using methanol as a blank. No color development is required for this determination process, and the HQ content in the VAM can be measured directly without any further processing. The limit of detection, limit of quantification, linearity range, accuracy, precision, robustness, and measurement uncertainty of this method have been measured and analyzed and found to be within the acceptable limit and range. The method shows linearity within 0.36–25.0 ppm HQ content in the solution range with a regression coefficient of 0.9999, a relative spike recovery of 101.35%, precision of 1.36%, relative bias of 0.55%, and robustness with a temperature variation of −5 °C.

Keywords: vinyl acetate monomer; hydroquinone; polymerization; UV-visible spectrophotometer

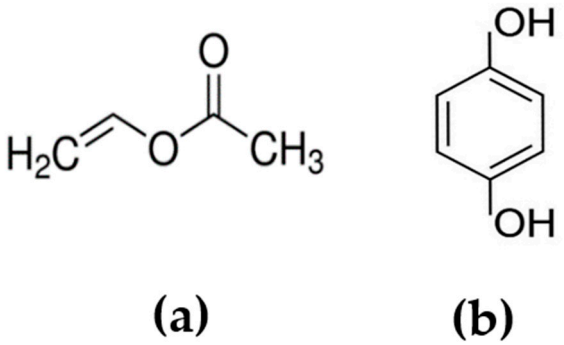
Citation: Rashid, M.A.M.; Rahman, M.; Mahmud, A.O.; Morshed, A.S.M.; Haque, M.M.; Hossain, M.M. UV-Vis Spectrophotometer as an Alternative Technique for the Determination of Hydroquinone in Vinyl Acetate Monomer. *Photochem* **2022**, 2, 435–447. https://doi.org/10.3390/photochem2020030

Academic Editors: Rui Fausto and Robert Kołos

Received: 16 May 2022
Accepted: 13 June 2022
Published: 15 June 2022

1. Introduction

Vinyl acetate monomer (VAM) may undergo a free radical chain polymerization phenomenon under constant temperature to produce polymers and copolymers used in water-based paints, adhesives, paper coatings, non-woven binders, and various applications at moderate temperatures [1–5]. The stability of VAM (Scheme 1a) depends mainly on the concentration of the inhibitor present in the VAM, the temperature of the storage vessel, and other surrounding conditions. The rapid spontaneous polymerization of VAM is the second most frequent cause of runaway reaction accidents in the chemical industry because of cross contamination [6]. Uncontrollable polymerization of VAM can occur due to improper handling, absence of inhibitor, inhibitor depletion for a prolonged storage, or the lack of proper storage precautions. Typically, hydroquinone (HQ) (Scheme 1b), having the chemical formula $C_6H_4(OH)_2$, is used in topical application in pharmaceuticals and as an inhibitor of the polymerization in VAM, including to prolong the shelf life of VAM for processing and safe transport and storage [7–9]. Most VAM shipped from the manufacturer should contain 3~5 ppm and up to 25 ppm HQ for regional shipments and for long-range shipments, respectively [8]. To avoid polymerization including cross contamination, the concentration of the HQ inhibitor should not decrease below a minimum effective level. Proper methods for accurate measurement of HQ content are highly desirable.



Scheme 1. Chemical structure of (**a**) VAM and (**b**) HQ.

There are several test methods for determining the HQ content in pharmaceutical products (e.g., cream), healthcare products (e.g., cosmetics), and various biological matrices by using UV, thin layer chromatography (TLC), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), and high-performance liquid chromatography (HPLC) [10–15]. Moreover, in the United State Pharmacopeia (USP) monograph, HQ has been determined in the pharmaceuticals cream by 1cm cells at the wavelength of maximum absorbance at about 293 nm, with a suitable spectrophotometer, using methanol as a blank [16]. In the Metler Toledo application note, HQ has been measured by UV spectrophotometer at about 289 nm using 0.05 (M) sulphuric acid as a diluent [17]. In the British Pharmacopeia (BP) monograph, Tretinoin, HQ, and Hydrocortisone have been determined in pharmaceuticals cream by using HPLC [Column-Waters Spherisorb ODS 1 (250 mm × 4.6 mm, 5 µm), Mobile Phase-Methanol: water (9:1, v/v), Diluent-Mobile Phase, Detection-UV, 295 nm] [18]. These methods are expensive, sophisticated, and need extensive experimental skills. Moreover, we did not find any study on determining the amount of HQ in the VAM using any of the above-mentioned methods. Thus, developing a simple, easy, cheap, and suitable validated technique to determine HQ content is timely right now.

To the best of our knowledge, only two test methods are available for the determination of the HQ content in VAM. They are the titrimetric method in ASTM D2193 (withdrawn standard without replacement) and the liquid chromatographic method in the Vinyl Acetate Safe Handling Guide [8,18]. However, these two methods require many reagents and sample processing for the analysis of the HQ content in the VAM; they are also time-consuming. In this contribution, a quicker and easier validated test method has been developed to determine the HQ content in the VAM by using the Ultra-Violet Visible (UV-Vis) spectrophotometer at absorbance of 293 nm and with methanol as a blank. Neither the color development nor the special reagent is required for this technique, and a VAM sample can be analyzed directly without further processing. After the development of the method, an extensive method validation was conducted to ensure the entire testing process.

2. Materials and Methods

2.1. Principle

The Beer–Lambert Law (also called Beer's Law), which is commonly applied to the measurements of chemical analysis, describes a relationship between the attenuation of light through a substance and the properties of the substance. The main principle of the law states that concentration and absorbance are directly proportional to each other [19,20]. The expression of the law is

$$A = k \times l \times c \quad (1)$$

where A is the absorbance, k is the proportionality constant, l is the path length, and c is the concentration of the absorbing chemical species.

2.2. Apparatus

(i) Spectrophotometer, absorbance at 293 nm; UV 1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan).
(ii) Volumetric Flask, 50 mL and 100 mL capacity.
(iii) Pipets or Auto-pipets, 1 mL or 2 mL.
(iv) Quartz cuvette; Material: Q, Path Length: 10 mm, Match Code: 6 (Starna Scientific Ltd., Ilford, UK).

2.3. Reagents

All the reagents and chemicals in this study were of high purity. HQ (Certified Reference Material: Catalogue no-N-12192-1G, Lot-10614100, Purity-99.4 ± 0.5%) was obtained from Chem Service Inc., West Chester, PA, USA. Methanol (Analytical Grade: Catalogue no-1.06009.2500, Lot-10983509 046, Purity-99.9%) was obtained from Merck, Germany. VAM (Batch-05000107470, Purity-99.98%) was collected from AIK MOH Paints & Chemicals Pte Ltd., Singapore.

2.4. Methods

2.4.1. Calibration

0.10 g of HQ was weighed into a 100-mL volumetric flask containing approximately 50 mL of Methanol. The mixture was stirred well until the solution was completed, then diluted to the mark with methanol. This was used as the stock solution of the HQ (1000 ppm). A series of standards were prepared by pipetting the 0.25 mL, 0.50 mL, 0.75 mL, 1.00 mL, and 1.25 mL portions of the HQ stock solution into respective 50 mL volumetric flasks. Each flask was diluted to the mark with methanol and mixed well. These standards contained approximately 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm of the HQ, respectively. The absorbance of each of these standards was determined at 293 nm using methanol as the blank. The calibration curve was constructed on rectangular coordinate graph paper or software by plotting the absorbance of the standards at 293 nm.

2.4.2. Solution for Validation

A precision and robustness solution (15 ppm) was prepared from the standard stock solution of the HQ by diluting 0.75 mL of the HQ stock solution in 50 mL volumetric flasks containing 30 mL methanol. The solution was mixed well and diluted to the mark with methanol. Following the same process, ten samples were prepared for each precision and robustness analysis. Robustness was analyzed by checking the temperature impact at 25 °C, 30 °C, and 35 °C. Solutions for accuracy (spike recovery) were prepared by using 0.10 mL of HQ stock solution with 30 mL VAM sample in a 50 mL volumetric flask. The solution was mixed well and diluted to the mark with VAM sample. Seven samples were prepared for the accuracy (spike recovery) analysis. Solutions for accuracy (bias) were prepared in such a way as to contain 80%, 100%, and 120% of the target concentration of 15 ppm (0.60 mL, 0.75 mL and 0.90 mL of HQ stock, respectively) in respect to 50 mL volumetric flasks. Seven samples were prepared for each concentration of accuracy (bias) solution.

2.4.3. Determination of HQ

No sample preparation was required or measured directly. The absorbance of the solution was determined at 293 nm using methanol as a blank. From the calibration curve, the HQ content was determined corresponding to the absorbance obtained. In this study, a Shimadzu UV-VIS 1800 double-beam spectrophotometer was used for analysis.

3. Result and Discussion

3.1. Determination of λ_{max} of HQ

To determine the maximum absorption (λ_{max}), 20 ppm standard solution of the HQ was prepared. Scanning of the HQ in a wavelength range from 200 nm to 400 nm showed

a λ_{max} at 293 nm (Figure 1). It is noted that there was no inference of methanol within this range.

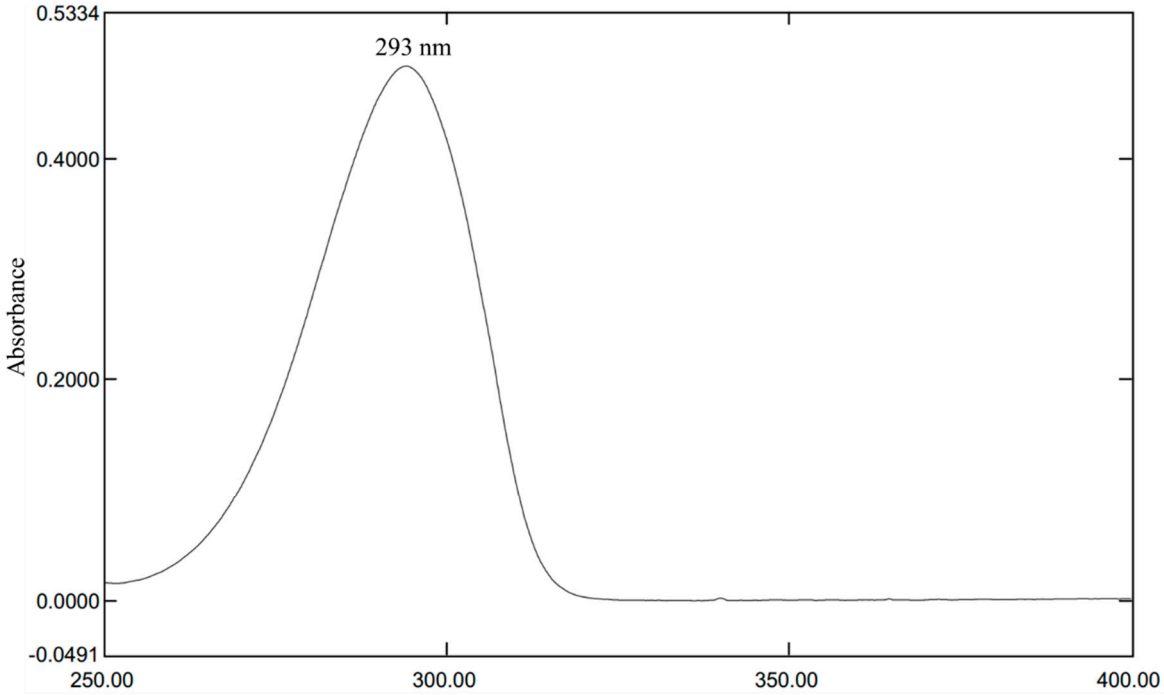


Figure 1. UV-Vis absorption spectra of the 20 ppm HQ standard solution within the range of 200~400 nm wavelength.

3.2. LOD (Limit of Detection)

LOD was analyzed with the lowest concentration of standard which can be detectable [21], as a blank sample has no response in the specified condition at 293 nm UV wavelength. A series of ten replicate measurements for the HQ content of 0.1 ppm and 0.2 ppm were analyzed, and a detectable response for 0.2 ppm was observed. The data of 0.2 ppm were used for the calculation of LOD as shown in Table 1. The LOD was found to be 0.14 ppm.

Table 1. Ten replicate measurements with respect to the theoretical concentration of the HQ at 0.2 ppm.

Absorbance of Sample	Experimental Concentration of HQ, ppm
0.005	0.142
0.006	0.167
0.005	0.106
0.005	0.116
0.005	0.131
0.005	0.115
0.005	0.118
0.005	0.125
0.005	0.123
0.005	0.120
Mean	**0.126**
Standard Deviation (SD)	**0.017**

LOD Calculation: Standard Deviation of LOD [22],

$$S' = SD \div \sqrt{n}; \quad (2)$$

For ten replicates, n = 10

$$\begin{aligned} \text{LOD} &= \text{LC}_{Mean} + 3S' \\ &= 0.126 + 3(0.017/\sqrt{10}) = 0.14 \text{ ppm} \end{aligned} \quad (3)$$

3.3. LOQ (Limit of Quantification)

LOQ data was analyzed with the lowest concentration of standard which can be quantifiable [21], as the blank sample had no response in the specified condition at 293 nm UV wavelength. A series of ten replicate measurements for the HQ content of 0.1 ppm, 0.2 ppm, and 0.3 ppm were analyzed, and a quantifiable response for 0.3 ppm was observed. The data of 0.3 ppm was used for the calculation of LOQ, which is presented in Table 2. The LOQ was found to be 0.36 ppm.

Table 2. Ten replicate measurements with respect to the theoretical concentration of 0.3 ppm HQ.

Absorbance of Sample	Experimental Concentration of HQ, ppm
0.006	0.264
0.007	0.316
0.007	0.293
0.007	0.293
0.006	0.271
0.006	0.267
0.008	0.325
0.007	0.318
0.007	0.289
0.007	0.290
Mean	**0.293**
Standard Deviation (SD)	**0.022**

LOQ Calculation: Standard Deviation of LOQ [22],

$$S' = SD \div \sqrt{n}; \quad (2)$$

For ten replicates, n = 10

$$\begin{aligned} \text{LOQ} &= \text{LC}_{Mean} + 10S' \\ &= 0.293 + 10(0.022/\sqrt{10}) = 0.36 \text{ ppm} \end{aligned} \quad (4)$$

3.4. Calibration Curve (Linearity and Range)

Under the optimum experimental conditions, a linear correlation was obtained between the UV-Vis absorbance and the HQ concentration within the range of 0.36~25 ppm. Concentrations of the standards against the respective absorbance were computed, and the linear regression curve was generated as shown in Figure 2. The regression coefficient R^2 = 0.9999 showed excellent linearity.

Regression line equation by the method of least squares $y = 0.0284x - 0.0005$

Regression coefficient, $R^2 = 0.9999$; slope = 0.0284 & y-intercept = 0.0005

3.5. Precision

3.5.1. Repeatability Data and Intermediate Precision Data

Analyst 1 was conducted repeatedly for determination of the HQ content by using ten samples (15 ppm standard solution). HQ content was measured from the corresponding absorbance for ten samples from the calibration curve. The percent of relative standard deviations (RSD) was calculated for analyst 1 using the following relationship:

$$\%\text{RSD} = \{(\text{Standard Deviation (SD)} \div \text{Mean (Avg.) Concentration}) \times 100\%\} \quad (5)$$

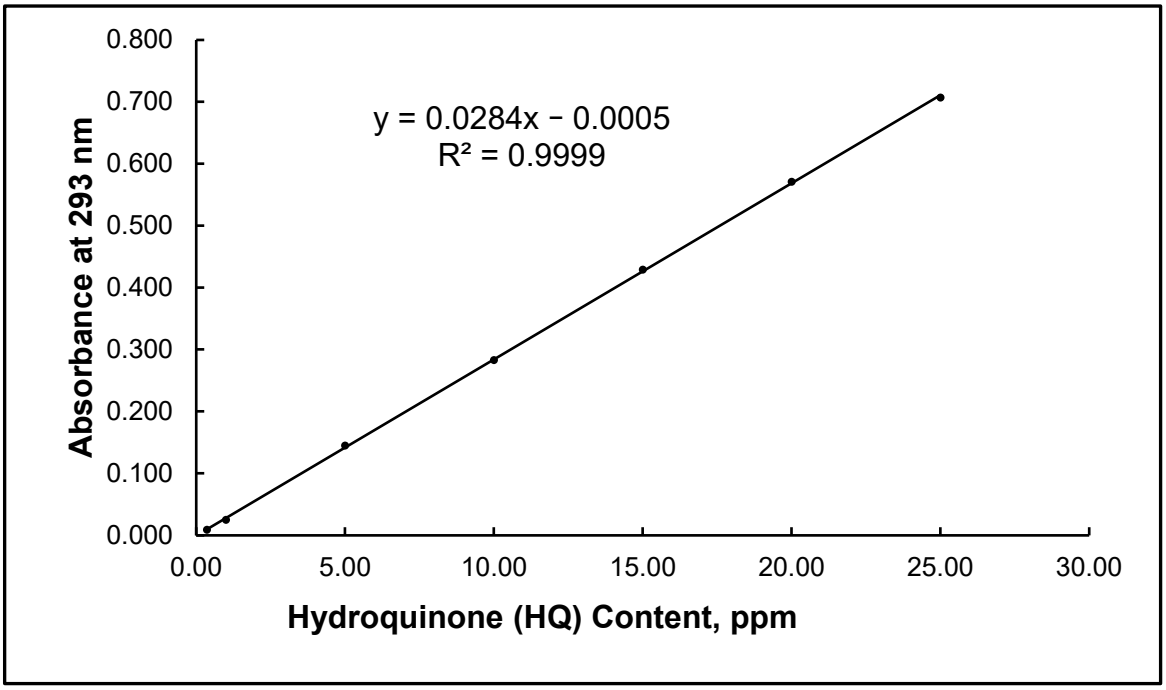


Figure 2. Linearity regression curve of UV-Vis absorbance and the HQ content within the range of 0.36~25 ppm.

The repeatability of the method was found to be 1.57%, whereas the acceptance criteria for the repeatability was 10% [23]. The percentage of RSD and is presented in Figure 3a. Separately, analyst 2 (second analyst) was measured with ten samples similarly to analyst 1. The HQ content was measured from the corresponding absorbance for each sample (15 ppm standard solution). The percentage of RSD was calculated for analyst 2 using the equation (5) and is shown in Figure 3b. The combination of the percentage RSD of analyst 1 and analyst 2 is called intermediate precision and is shown in Table 3. The intermediate precision of the method was found to be 1.36%, whereas the acceptance criteria for repeatability was 10% [24], ensuring that the repeatability and precision of this method are acceptable beyond a doubt.

Table 3. Intermediate precision data from 20 samples of 15 ppm HQ solution.

Content	Result
Average Content of HQ (ppm) of 20 Nos	15.14
Standard Deviation (SD) of 20 Nos	0.21
%RSD of 20 Nos measurements	1.36%

3.5.2. Inter-Laboratory Comparison (Reproducibility Test)

An inter-laboratory comparison was conducted for the reproducibility test with the following research laboratory. LAB A: Research & Development Center, Berger Paints Bangladesh Limited, Dhaka-Bangladesh; LAB B: Leather Research Institute, Bangladesh Council of Scientific and Industrial Research, Dhaka-Bangladesh; and LAB C: Wazed Miah Science Research Centre (WMSRC), Jahangirnagar University, Dhaka-Bangladesh. The data obtained from the three laboratories were shown in Table 4. The mean value of the HQ content was 15.37 ppm, which was very similar to our study, thus ensuring a satisfactory reproducibility for our method.

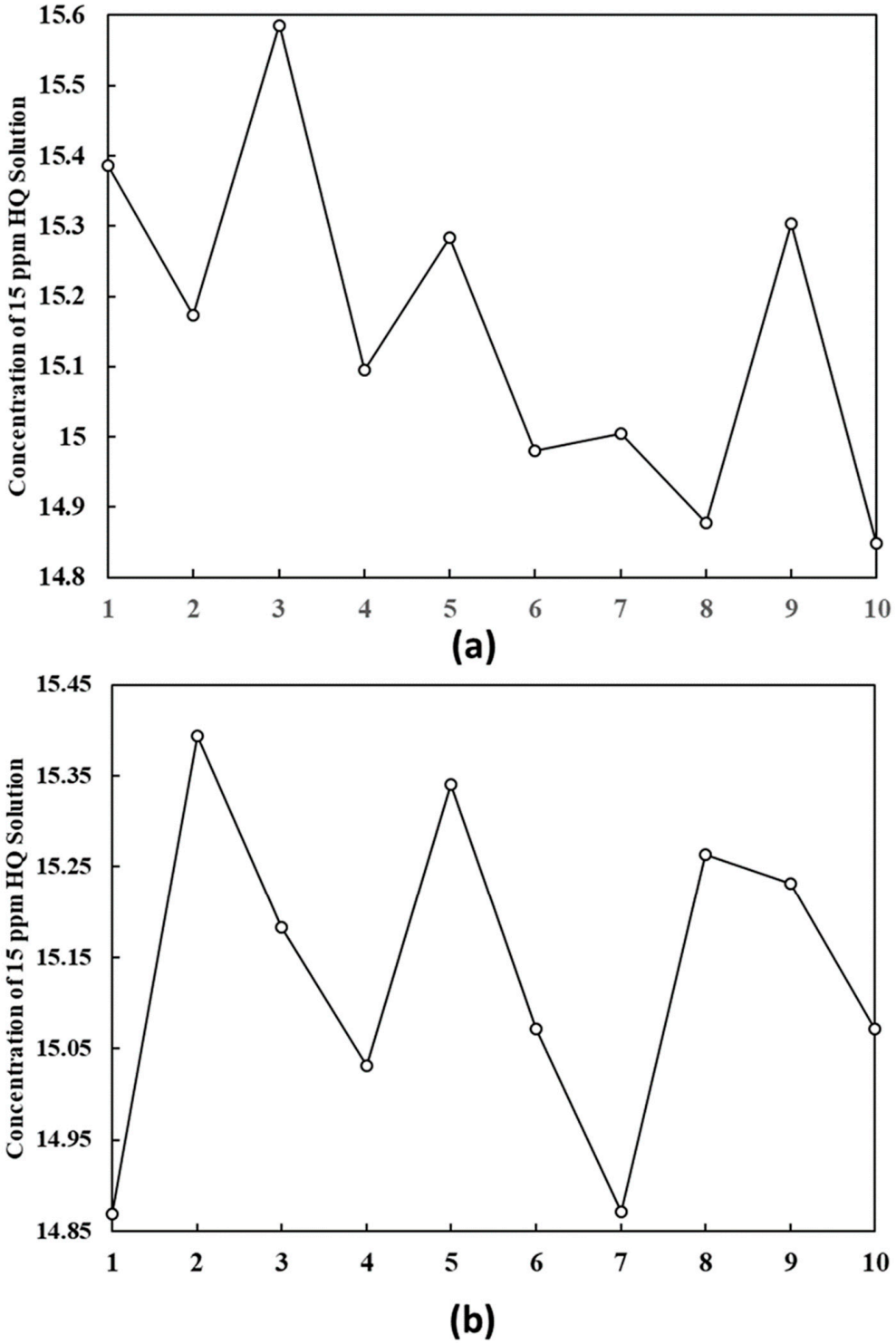


Figure 3. (**a**) Repeatability data for analyst 1 and (**b**) Intermediate precision data for analyst 2 for 15 ppm HQ content.

Table 4. Inter-laboratory comparison data of the reproducibility test.

Participant Laboratory	Obtained Result, ppm	Z Score = (Lab Result—Mean)/SD	Remarks
LAB A	15.44	0.167	Satisfactory
LAB B	15.75	0.906	Satisfactory
LAB C	14.92	−1.073	Satisfactory
Mean value	**15.37**	Acceptance Criteria: Z score ≤ 2.0	
Standard Deviation	**0.4194**		

3.6. Accuracy

3.6.1. Accuracy Data (Percentage of Spike Recovery)

Accuracy data were measured by adding known amounts of analyte to the sample solution (7 NOS solution samples prepared) and have been listed in Table 5. The concentration values were calculated from the corresponding absorbance for the sample and the spiked sample. The percentage of spike recovery was calculated using the following equation [24]:

$$\% \text{ Spike Recovery} = [\{(\text{Mean Value Spiked Sample} - \text{Mean Value Sample}) \div \text{Spiked Concentration}\} \times 100] \quad (6)$$

Table 5. Accuracy data (percentage of spike recovery in sample) with respect to spiked concentration 2 ppm.

Sample Details	Absorbance	Experimental Concentration	Mean Value	% Spiked Recovery *
Sample	0.438	15.382	15.372	Spike recovery of Hydroquinone = (17.399 − 15.372)/2 =101.35%
	0.438	15.381		
	0.437	15.366		
	0.437	15.371		
	0.437	15.363		
	0.438	15.372		
	0.437	15.370		
Spiked Sample	0.492	17.287	17.399	
	0.487	17.102		
	0.502	17.650		
	0.500	17.561		
	0.494	17.379		
	0.495	17.385		
	0.494	17.430		

* Acceptance criteria: the accuracy data (percentage of spike recovery) should be between −20.0% and 10.0% [25].

It is seen from Table 5 that the spike recovery of the HQ was found to be 101.35%, suggesting that this method is highly accurate for the determination of HQ content in VAM.

3.6.2. Bias

Bias samples were analyzed by adding known amounts of analyte to the blank solution at various concentrations, and three different concentrations of HQ (7 NOS samples for each concentration) were prepared. Concentration values were measured from the corresponding absorbance for the three concentrations [26]. The relative percentage of bias was calculated using the following formula and is listed in Table 6.

$$\% \text{ Bias} = [\{(\text{Mean for Experiment data} - \text{Theoretical Concentration}) \div \text{Theoretical Concentration}\} \times 100] \quad (7)$$

Table 6. The results of percentage of bias.

Theoretical Concentration, ppm	Absorbance	Experimental Concentration, ppm	Mean Value	% Bias
12	0.353	12.400	12.086	0.72%
	0.347	12.182		
	0.341	11.957		
	0.338	11.860		
	0.341	11.966		
	0.345	12.091		
	0.346	12.147		
15	0.430	15.090	15.173	1.16%
	0.425	14.928		
	0.427	14.995		
	0.432	15.170		
	0.438	15.397		
	0.440	15.443		
	0.432	15.190		
18	0.506	17.798	17.959	−0.23%
	0.517	18.187		
	0.521	18.302		
	0.508	17.857		
	0.508	17.863		
	0.505	17.744		
	0.511	17.960		
			Mean	**0.55%**

The percentage of bias of the HQ was found to be 0.72%, 1.16%, and −0.23% for 12 ppm, 15 ppm, and 18 ppm, respectively. The relative (mean) bias was observed as 0.55%. The acceptance criteria of the accuracy data (percentage of spike recovery and bias) should be between −20.0% and 10.0% [27]. This confirms the accuracy of our method.

3.7. Robustness

Robustness data for this method were analyzed by maintaining a 5 °C temperature difference in samples of 15 ppm HQ standard solution. Single absorbance was measured for each of ten samples against 25 °C, 30 °C, and 35 °C temperature, respectively. The percentage of RSD was calculated for each temperature variance using the following relationship [28]:

$$\%RSD = [(\text{Standard Deviation (SD)} \div \text{Mean (Avg.) Concentration}) \times 100\%] \quad (8)$$

Figure 4 represents the robustness of the method, which was 0.83%, 0.73%, and 1.37% at 25 °C, 30 °C, and 35 °C temperature, respectively.

3.8. Measurement Uncertainty (MU)

MU is the expression of the statistical dispersion of the values attributed to a measured quantity. For the calculation of MU data, it is necessary to consider type A source (obtained from repeatability data) and type B source (obtained from certificate), which together represent the range of values that can reasonably be attributed to the quantity being measured. Type A source for MU data calculation was obtained from 10 replicate measurements. The repeatability of 10 (15 ppm HQ standard solution) test samples was determined by obtaining the type A uncertainty (Mean/$\sqrt{n}$, n = number of replicates), which has been represented in Figure 5. The expanded MU has been measured and listed in Table 7. It is observed from Figure 5 and Table 7 that the expanded MU of the method was found to be 15.15 ± 0.656 ppm and that the percentage of expanded uncertainty was 4.33% with a 95% confidence level. The following sources were considered in MU calculation: (standard uncertainty of type B obtained from certificate).

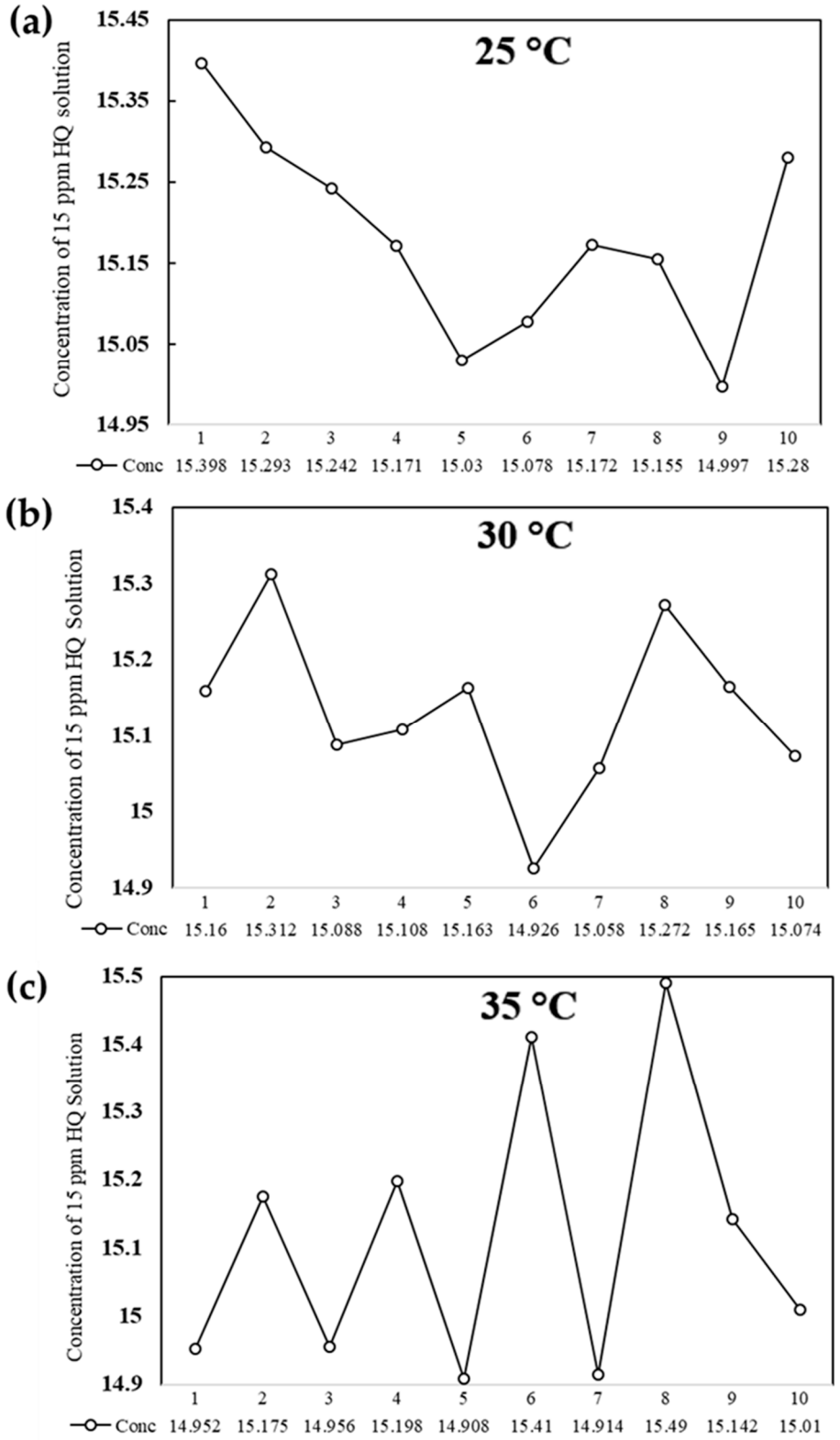


Figure 4. Robustness data at (**a**) 25 °C, (**b**) 30 °C, and (**c**) 35 °C temperature.

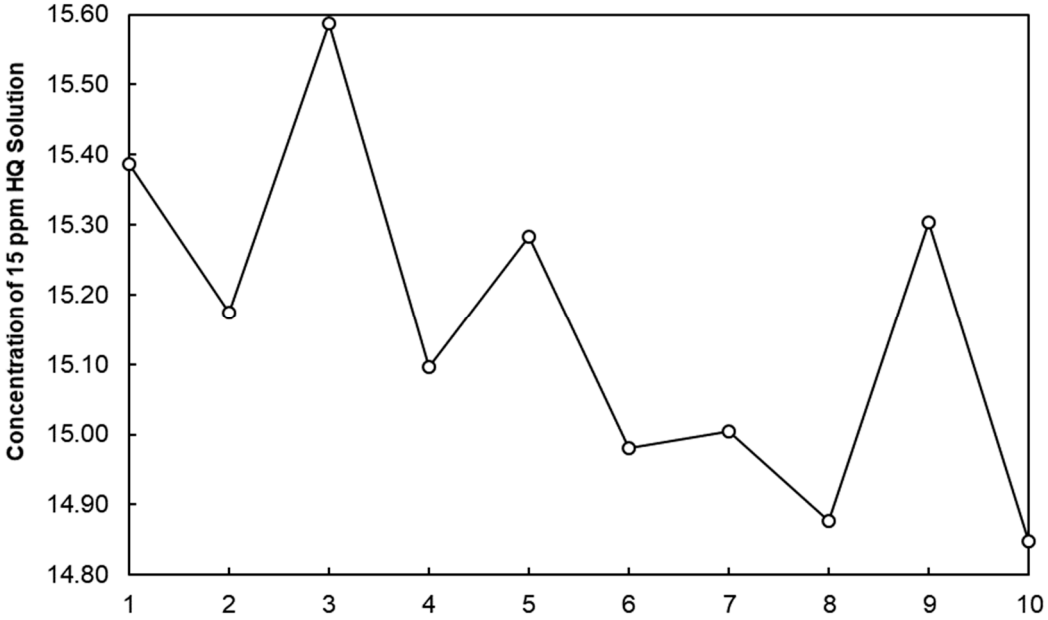


Figure 5. Measurement uncertainty data from type A source for ten standard HQ test samples.

Table 7. Expanded measurement uncertainty.

Sources of Uncertainty	Standard Uncertainty, δ	Divisor, x	Relative Uncertainty
Repeatability	0.0751	15.15	0.0050
Volumetric Flask	0.0300	50.00	0.0006
Balance	0.0010	0.0500	0.0200
Auto Pipette	0.0004	1.000	0.0004
Purity of CRM	0.2887	99.40	0.0029
Recovery %	−0.5931	100.00	−0.0059
Linearity curve	0.0001	1.00	0.0001
	combined relative uncertainty		0.022
	expanded uncertainty		0.656
	% expanded uncertainty		4.33

Type B Source:

- Balance (uncertainty: 0.0010 g)
- Volumetric Flask (uncertainty: 0.0300 mL)
- Pipette (uncertainty: 0.0004 mL)
- Recovery (uncertainty: −0.5931)
- − Certified reference material (uncertainty: 0.2887)
- Calibration curve linearity (uncertainty: 1−0.9999 = 0.0001)

$$\text{Combined relative uncertainty} = \sqrt{\{(\delta 1/x1)2 + (\delta 2/x2)2 + \ldots \ldots + (\delta n/xn)^2\}} \quad (9)$$

where δ = standard uncertainty of source, x = divisor, and n = number of source

$$\text{Expanded uncertainty} = \text{combined relative uncertainty x K x mean of type A data} \quad (10)$$

where coverage factor K = 2 for 95% confidence level

$$\text{Percentage of expanded uncertainty} = \text{expanded uncertainty} \times 100/\text{Mean of type A data} \quad (11)$$

4. Conclusions

In this study, an alternative test method was developed for determining the HQ content in VAM by using a UV-Vis spectrophotometer. This method does not require

further sample processing or color development and excludes only methanol as reagent for standard HQ solution preparation. LOD, LOQ, linearity range, accuracy (spike recovery and bias), precision (repeatability, intermediate precision, and reproducibility), robustness under 5 °C temperature variation, and MU were found to be within the acceptable limit and required range. Therefore, this method has been extensively validated, and we believe that it will be very suitable for the accurate determination of the HQ content in VAM using a UV-Vis spectrophotometer. As the determination of the HQ content in VAM is a crucial factor for reducing unexpected polymerization for ensuring proper storage and transportation, industries and suppliers need an easy and quick method for measuring HQ content. Thus, this validated test method will be very useful to manufacturers and suppliers of VAM to ensure quality and give sustainability during storage and transportation.

Author Contributions: Writing—review & editing & Methodology, M.A.M.R.; Writing—original draft, Data curation, Formal analysis & Investigation, M.R.; Supervision & Funding acquisition, A.O.M.; Conceptualization & Project administration, A.S.M.M.; Formal analysis & Validation, M.M.H. (Md Masudul Haque); Resources & Validation, M.M.H. (Md Mobarak Hossain). All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by Berger Paints Bangladesh Limited, Dhaka, Bangladesh.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This experiment was conducted mainly at the laboratory of the Research and Development Center, Berger Paints Bangladesh Limited. The inter-laboratory testing comparison was conducted at the government-owned laboratory of the Leather Research Institute, Bangladesh Council of Scientific and Industrial Research and at the Wazed Miah Science Research Centre (WMSRC), Jahangirnagar University.

Conflicts of Interest: The authors declare no conflict of interest.

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