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Validation of a UV-VIS-NIR Spectrophotometric Method for Determination of Sodium Benzoate in Water[§]

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Abstract: Sodium benzoate is a widely used food preservatives and its determination receives much interest. This article describes the development and validation of a method using a UV-VIS-NIR Spectrophotometer at 225 nm to determine the mass fraction of sodium benzoate in water for the purpose of characterization of a reference material. The study was carried out in accordance with the ICH and EURACHEM guides using a primary reference material of sodium benzoate of purity 99.98±0.22%. The studied performance characteristics were the limit of detection (LOD), limit of quantification (LOQ), selectivity, linearity, accuracy, precision, recovery and bias. The results obtained were statistically analyzed and the method showed very good linearity in the selected calibration range. The LOD and the LOQ were found 0.19 and 0.57 mg/kg respectively. This method demonstrated very good accuracy ranging from 99.54 to 100.08% and precision, 0.39% RSD, signifying its high reliability in producing precise and accurate results. The validation results also revealed that the method is fit-for-the purpose of determination of sodium benzoate in water.

Keywords: Sodium benzoate; UV-VIS-NIR spectrophotometer; validation; linearity; precision; accuracy.

1. Introduction

Sodium benzoate is a widely used food preservative, valued for its ability to inhibit microbial growth and extend shelf life in numerous products, ranging from beverages and jams to condiments and salad dressings [1,2]. Due to its safety and efficacy, regulatory bodies worldwide have approved it. However, accurate and reliable quantification of sodium benzoate in diverse food matrices is crucial to ensure consumer safety and compliance with regulations [3,4]. The UV-VIS-NIR spectrophotometry, a common analytical technique, has been employed for sodium benzoate determination due to its simplicity, affordability and availability. Numerous previous studies have utilized this technique, measuring the absorbance of sodium benzoate solutions at its characteristic wavelength, typically around 225-230 nm [5-8]. Given the critical nature of accurate sodium benzoate measurement in ensuring food

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safety and quality, proper method validation is essential to establish the suitability of the method for its use. The method validation ensures that generated data possess the necessary characteristics of the limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision, selectivity and bias [9]. Review of the literature reveals that no validation of measuring sodium benzoate in pure water has been reported. This study aims to address this gap for the purpose of characterization of a reference material solution using UV-VIS-NIR spectrophotometry. To avoid uncertainties caused by volume measurements, the validation results were expressed in mg/kg. The calibration range of the method was selected 5-50 mg/kg, middle of which, the mass fraction of the reference material to be analyzed lies. The key performance characteristics that will be studied are: LOD, LOQ, linearity, accuracy, precision, recovery and bias in accordance with the ICH and EURACHEM guides [10,11,12]. The definition of each of these performance characteristics and its meaning are well explained in both guides and the study will focus on the realization of these meanings. By establishing a well-validated method and reporting its performance characteristics transparently, this study will contribute to a reliable and accurate sodium benzoate analysis, ultimately supporting food safety and quality control.

2. Materials and methods

The primary reference material used for calibration of the UV-VIS-NIR Spectrophotometer was of purity 99.997 ± 0.232% measured by qNMR at UME, Türkiye. Ultrapure water was obtained from Millipore Milli-Q RG, USA. The sodium benzoate CRM (99.7 ± 0.2%) used as a sample for study was provided by UME, Türkiye. The UV-VIS-NIR spectrophotometer used was Hitachi UH4150, Japan with an automatic wavelength correction and a pair of 1 cm matched quartz cells. The spectral band width was 1 nm and the wave length accuracy was 0.3 nm. Sodium benzoate samples were measured in aqueous solution at 225 nm. The preparation of the calibration solutions was carried out as described elsewhere [1,5].

3. Results and Discussion

3.1. Limits of Detection and Quantification (LOD and LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined in two stages. First, the mass fraction at which the behavior of sodium benzoate starts to follow the Beer-Lambert Law was identified. This mass fraction is the one at which, the absorbance starts to increase as the mass fraction of the measured sample increases. For this step, seven sodium benzoate mass fractions were gravimetrically prepared (0.0001, 0.0005, 0.001, 0.02, 0.05, 0.1, 0.15 and 0.25 mg/kg) and the absorbance of each was measured ten times as reported in Table 1. From this table, it can be seen that in the mass fraction range, 0.0001 to 0.02 mg/kg the absorbance decreases as the mass fraction increases, i.e. the behavior of sodium benzoate in this region is not obeying Beer-Lambert Law. However, mass fractions from 0.05 to 0.25 mg/kg displayed an increasing absorbance with the increase of the mole fraction in a clear adherence to the Beer-Lambert Law. Therefore, this range (0.05-0.25) was used to determine both LOD and LOQ based on the approach of standard deviation of the response and the slope of the calibration curve as indicated by ICH [10].

A calibration curve was constructed using three mass fractions (0.05, 0.15 and 0.25 mg/kg) against their corresponding average absorbance values (0.0033, 0.016 and 0.023) and the obtained calibration function was y = 0.0965x - 0.0005. To determine the LOD and LOQ, a sample of sodium benzoate with a mass fraction of 0.12 mg/kg was prepared, and its absorbance measured ten times. The corresponding mass fractions were calculated using the calibration function and were recorded in Table 2. The LOD and LOQ were calculated using equations 1 and 2

$$LOD = \frac{\sigma}{S} \times 3.3 \qquad (1)$$

$$LOQ = \frac{\sigma}{S} \times 10 \qquad (2)$$

where
σ - is the standard deviation of the response
S - the slope of the calibration curve

The obtained values were found 0.19 and 0.57 mg/kg respectively [10].

Table 1. Absorbance values of seven mass fractions for LOD and LOQ determinations

Mass fraction (mg/kg)	**0.0001**	**0.0005**	**0.001**	**0.02**	**0.05**	**0.15**	**0.25**
Absorbance	0.033	0.012	0.014	0.014	0.002	0.016	0.023
	0.032	0.012	0.014	0.014	0.002	0.016	0.022
	0.033	0.012	0.014	0.014	0.002	0.016	0.022
	0.033	0.012	0.014	0.015	0.003	0.016	0.022
	0.033	0.012	0.014	0.014	0.004	0.016	0.023
	0.033	0.012	0.015	0.014	0.004	0.016	0.022
	0.033	0.012	0.014	0.014	0.003	0.016	0.023
	0.033	0.012	0.014	0.015	0.004	0.016	0.023
	0.033	0.012	0.014	0.015	0.004	0.016	0.023
	0.033	0.012	0.014	0.014	0.005	0.016	0.023

Table 2. Absorbance and the corresponding mass fraction values of the o.12 mg/kg sample

Abs	**Slope (a)**	**Intercept (b)**	**C (mg/kg)**	$\bar{x}$	**SD**
0.012	0.0965	-0.0005	0.13		
0.012	0.0965	-0.0005	0.13		
0.012	0.0965	-0.0005	0.13		
0.013	0.0965	-0.0005	0.14		
0.012	0.0965	-0.0005	0.13	0.13	0.0055
0.013	0.0965	-0.0005	0.14		
0.012	0.0965	-0.0005	0.13		
0.013	0.0965	-0.0005	0.14		
0.013	0.0965	-0.0005	0.14		
0.013	0.0965	-0.0005	0.14		

3.2. Selectivity

The selectivity of a method refers to its ability to precisely measure a specific compound without interference from other compounds in the sample [13]. To evaluate selectivity, a blank sample was spiked with sodium benzoate and analyzed by the UV-VIS-NIR spectrophotometer to produce the absorption spectrum shown in Figure 1.

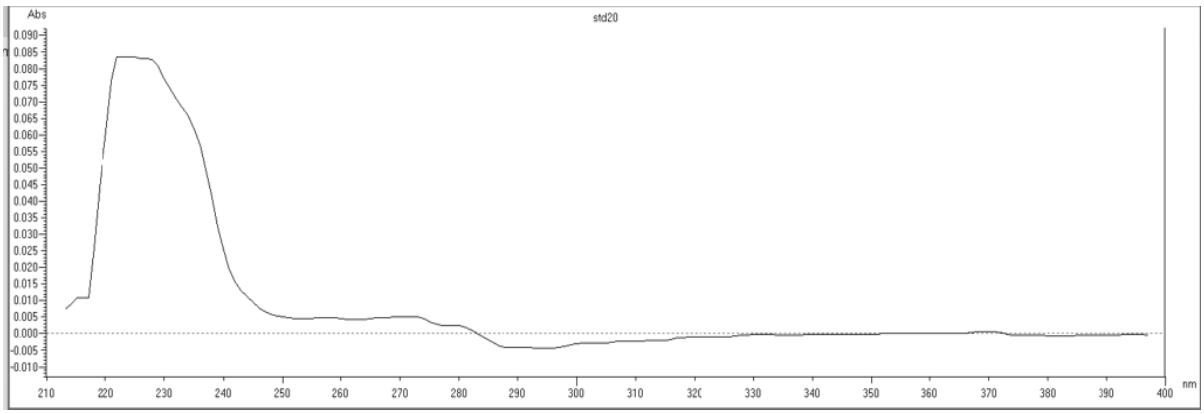

Figure 1. The UV absorption spectra of sodium benzoate at 225 nm

From this Figure, it can be noticed that, no other absorption peaks appeared around 225 nm, where sodium benzoate absorbs the UV light. This suggests high selectivity for this method in analyzing sodium benzoate.

3.3. Linearity

Six calibration mass fraction levels were prepared at 5.07, 10.11, 20.13, 30.14, 40.07, and 50.23 mg/kg, and each level was measured five times. The calibration line was generated by plotting the average absorbance (*y*-axis) against the corresponding mass fraction levels (*x*-axis), as shown in Figure 2.

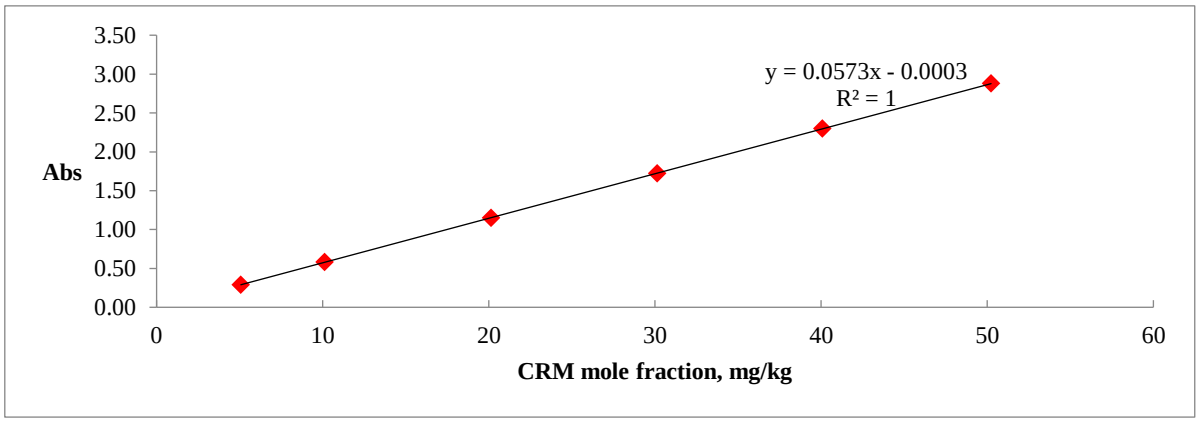

Figure 2. The calibration line of the UV-VIS-NIR spectrophotometer

Visual inspection of the line reveals a direct proportional relationship between the change in absorbance and the change in mass fraction. The equation for this linear calibration is y = 0.0573x - 0.0003, with a near-zero intercept. For further assessment of linearity, the theoretical values (ŷ) of all the calibration points were calculated using the calibration function. The residuals, defined as the difference between the measured absorbance (y) and the corresponding theoretical value (ŷ), were then calculated. The calculated residuals (y- ŷ) were found randomly distributed around zero as illustrated in Figure 3. This indicates excellent linearity of the method according to the IUPAC Recommendations 1998 [13].

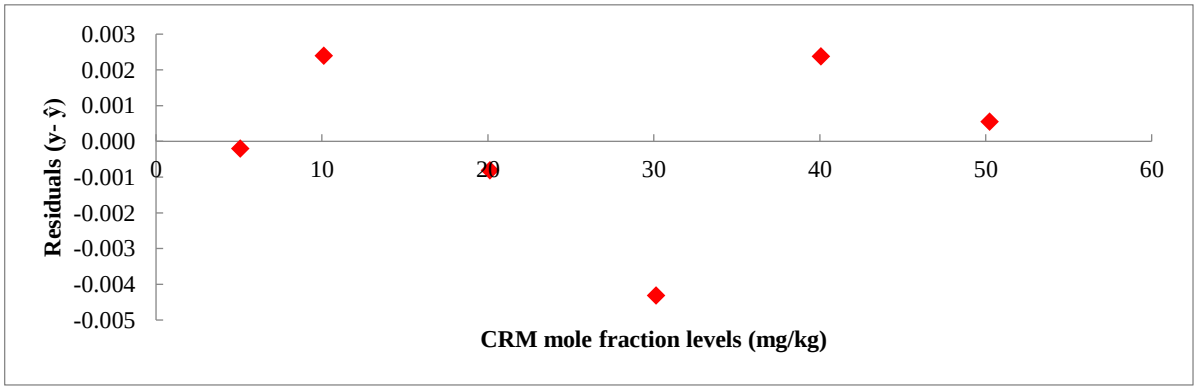

Figure 3. Residuals of the calibration points around an axis 0

3.4. Accuracy

The method accuracy was evaluated at three CRM mass fractions: low (5 mg/kg), medium (30 mg/kg) and high (50 mg/kg). Each level was measured seven times, and the measured absorbance values were presented in Tables 3, 4 and 5 respectively. Using the calibration equation ($y = 0.0573x - 0.0003$),

the mass fraction of each measured sample, x_i was calculated and recorded in the tables. Equation 3 was then employed to determine the % accuracy and the results were also shown in Tables 3, 4 and 5.

$$\% \, Accuracy = \frac{x_i}{x_{CRM}} \, x \, 100 \tag{3}$$

Table 3. Accuracy (%) study at low level (5 mg/kg)

y **(Abs)**	***a***	***b***	***y-b***	x_i	x_{CRM}	**%Accuracy**
0.289	0.0573	-0.0003	0.2893	5.0489	5.07	99.540
0.289	0.0573	-0.0003	0.2893	5.0489	5.07	99.540
0.289	0.0573	-0.0003	0.2893	5.0489	5.07	99.540
0.289	0.0573	-0.0003	0.2893	5.0489	5.07	99.540
0.289	0.0573	-0.0003	0.2893	5.0489	5.07	99.540
0.289	0.0573	-0.0003	0.2893	5.0489	5.07	99.540
0.289	0.0573	-0.0003	0.2893	5.0489	5.07	99.540

Table 4. Accuracy (%) study at medium level (30 mg/kg)

y **(Abs)**	***a***	***b***	***y-b***	x_i	x_{CRM}	**%Accuracy**
1.726	0.0573	-0.0003	1.7263	30.1274	30.14	99.96
1.727	0.0573	-0.0003	1.7273	30.1449	30.14	100.02
1.726	0.0573	-0.0003	1.7263	30.1274	30.14	99.96
1.726	0.0573	-0.0003	1.7263	30.1274	30.14	99.96
1.727	0.0573	-0.0003	1.7273	30.1449	30.14	100.02
1.727	0.0573	-0.0003	1.7273	30.1449	30.14	100.02
1.728	0.0573	-0.0003	1.7283	30.1623	30.14	100.08

Table 5. Accuracy (%) study at high level (50 mg/kg)

y **(Abs)**	***a***	***b***	***y-b***	x_i	x_{CRM}	**%Accuracy**
2.877	0.0573	-0.0003	2.8773	50.2496	50.23	100.04
2.879	0.0573	-0.0003	2.8793	50.2321	50.23	100.01
2.878	0.0573	-0.0003	2.8783	50.1798	50.23	99.90
2.875	0.0573	-0.0003	2.8753	50.1798	50.23	99.90
2.875	0.0573	-0.0003	2.8753	50.1798	50.23	99.90
2.875	0.0573	-0.0003	2.8753	50.2147	50.23	99.97
2.877	0.0573	-0.0003	2.8773	50.2147	50.23	99.97

An analysis of the results in Tables 3, 4 and 5 reveals that the method exhibits excellent accuracy in the three levels, ranging from 99.54% to 100.08% [9,11,12]. This high accuracy demonstrates the method suitability for the intended measurements of sodium benzoate in water by UV-VIS-NIR spectrophotometer.

3.4.1. Recovery and Bias

Recovery reflects the closeness of the measured value to a known reference value, typically expressed as a percentage. Ideally, recovery should be as close to 100% as possible, indicating that the measurements accurately represent the actual concentration in the sample. On the other hand, bias represents the systematic difference between the average measured value and the known reference value [11]. A positive bias signifies overestimation, while a negative bias indicates underestimation. It is crucial to note that even with high recovery, a significant bias can still lead to inaccurate results. To evaluate this parameter, a sample of CRM of mass fractions (25.15 mg/kg) was measured ten times using the UV-VIS-NIR Spectrophotometer and the measurement results were presented in Table 7.

Table 7. The 10 measured absorbance results and the corresponding mass fractions (mg/kg)

Abs	Mass fraction (mg/kg)
1.445	25.20
1.445	25.20
1.446	25.21
1.446	25.21
1.446	25.21
1.446	25.21
1.446	25.21
1.447	25.23
1.446	25.21
1.446	25.21
$\bar{x}$	25.21

The average value, $\bar{x}$ was calculated to be 25.21 mg/kg. Subsequently, the recovery was determined by Equation 4 and was found to be 100.24%.

$$R(\%) = \frac{\bar{x}}{x_{CRM}} \; x \; 100 \qquad (4)$$

This analysis demonstrates that the UV-VIS-NIR spectrophotometry method exhibits excellent recovery, indicating close agreement between the measured and the actual mass fractions. However, further evaluation of bias is necessary to ensure the absence of systematic overestimation or underestimation, which could potentially compromise the accuracy of the measurements. The bias was studied as the difference between average of the measured values and value of the CRM. Equation 5 was used for this calculation and a bias of 0.06 mg/kg was found. This bias was also calculated as percentage using equation 6 and was found 0.024%.

$$b = \bar{x} - x_{ref} \qquad (5) \qquad b(\%) = \frac{\bar{x} - x_{ref}}{x_{ref}} \qquad (6)$$

The calculated bias was evaluated whether statistically significant. If it is found significant, a correction factor is applied to the results. Conversely, if it is found non-significant, means that the method is free from bias. This evaluation was based on the criterion in Equation 7, where the standard deviation σ was calculated using Equations 8. It involves the calculated values of Sb and Sr (mg/kg) shown in Table 8 and the standard uncertainty of the CRM (u_{CRM}), 0.22% [17,18,19].

$$-2\sigma \le b \le +2\sigma \qquad (7) \qquad \sigma = \sqrt{\left(S_b\right)^2 + \left(\frac{S_r}{n}\right)^2 + \left(u_{CRM}\right)^2} \qquad (8)$$

As the uncertainty contributions are of different units, the relative uncertainty was chosen as the appropriate approach for calculation of σ. This involved dividing both S_b and S_r/n by the average calculated mole fraction and uncertainty of the purity by the CRM purity (%) value. The square root of the sum of squares was then multiplied by the measured mole fraction to obtain σ as 0.117 mg/kg. Examining the bias value, it is evident that it satisfies the criteria outlined in Equation 9, which leads us to conclude that the method does not exhibit any significant bias.

3.5. Precision

To evaluate the precision of the method, three analysts, analyzed a sample of 25 mg/kg ten times each and the obtained mass fractions were recorded in Table 8.

Table 8. Results of the precision study in mg/kg

	Analyst 1	Analyst 2	Analyst 3
	25.20	25.12	25.31
	25.20	25.12	25.31
	25.21	25.12	25.31
	25.21	25.12	25.33
	25.21	25.12	25.31
	25.21	25.12	25.31
	25.21	25.12	25.33
	25.23	25.12	25.33
	25.21	25.12	25.31
	25.21	25.12	25.33
$\bar{x}$	25.21	25.12	25.32
X_G		25.22	
S_b		0.099	
S_r		0.0076	
S_I		0.099	
% RSD		0.39	

Precision was evaluated as the intermediate precision, S_I by combining the standard deviation between analysts (s_b) and the standard deviation of the repeatability (s_r). The standard deviation between analysts, s_b was calculated according to Equation 9. The *MSb* , *MSw* are the mean squares between and within groups (Table 9) and *n* is the number of measurements [11,14-16].

$$S_b = \sqrt{\frac{MS_b - MS_w}{n}} \qquad (9)$$

Table 9. ANOVA table

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.19656	2	0.09828	1701	3.97E-29	3.35413
Within Groups	0.00156	27	5.78E-05			
Total	0.19812	29				

To calculate the standard deviation of repeatability (S_r), Equation 10 was used.

$$S_r = \sqrt{MS_w} \qquad (10)$$

The intermediate precision (S_I) was then determined using Equation 11.

$$S_I = \sqrt{(s_b)^2 + (s_r)^2} \qquad (11)$$

Equation 12 was used to calculate the precision as the percentage relative standard deviation (%RSD) by dividing S_I over the grand mean (X_G). The resulting value of 0.39% is small and clearly indicates a satisfactory precision of the method.

$$\%\ RSD = \frac{S_I}{X_G} \times 100 \qquad (12)$$

4. Conclusion

A reliable method for determining the mass fraction of sodium benzoate in water using a UV-VIS-NIR Spectrophotometer at 225 nm has been developed and validated. The validation was based on the ICH and EURACHEM guidelines. The method demonstrated excellent linearity within the chosen

range (5-50 mg/kg) and very clear selectivity to sodium benzoate. The limits of detection (LOD) and quantification (LOQ) were established at 0.19 mg/kg and 0.57 mg/kg, respectively. The method proved to be free from any significant bias, exhibited very good accuracy (99.54-100.08%) and precision (0.39% RSD) signifying its ability to produce precise and accurate results. These validation results confirm the method suitability for determining sodium benzoate in water, particularly for characterizing reference materials.

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