Simultaneous Determination of Binary Mixtures of Aniline and 2-Nitronailine in Tap Water Samples by Derivative Spectrophotometry

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Abstract: This paper describes a simple, rapid, and reliable derivative spectrophotometric technique for the simultaneous determination of aniline and 2-nitroaniline in tap water samples. The measurement method for first and second derivative spectrophotometry was based on the zero-crossing technique. The calibration graphs are in the concentration spectrum, linear of 1.0 – 20.0 µgmL^{-1} and 1-11.0 µgmL^{-1} for aniline and 2-nitroaniline successively. Relative standard deviations were less than 4.7% and 4.83%. The detection limits were in the range of 0.03-0.062 µg /mL and the limits of quantification were in the range of 0.25-0.57 µgmL^{-1}, respectively. The recoveries range from 97.5 – 101.66 % for aniline and 96.67–102.22 % for 2-nitroaniline. The values of r^{2}of the method were 0.9991 and 0.9999, correspondingly. The proposed methods are successfully applied to estimate binary mixtures of aniline and 2-nitroaniline in tap water samples.

Key words: Aniline, 2-Nitroaniline, Derivative Spectrophotometry, Tap Water Samples

1. Introduction

Aniline (AN) is a chemical compound with the formula C_6H_5NH. It is a typical simple aromatic amine with an amino group attached to a phenyl group in one position. (Anjalin et al., 2020). Aniline is mainly utilized in the production of precursors for polyurethane and other industrial chemicals. Aniline, like most volatile amines, has a fishy odor (Thomas et al., 2007). AN and its derivatives are used as intermediates in the synthesis of dyes, pharmaceuticals, ,pesticides, polymers (including polyurethane and rubber additives),and photographic chemicals, as well as such as precursors for amino aromatic derivatives (Khan et al., 2009, Sihtmäe et al., 2010, Jiang et al., 2016). Aniline and other amines could exist in the environment as a result of industrial discharge from factories that use anilines as intermediates or as an outcome of the decomposition of certain herbicides (Zhu et al., 2002).

2-Nitroaniline (2-NAN) is an organic compound with the formula $C_6H_6N_2O_2$. It's a massive orange solid at room temperature. 2-Nitroaniline is an environmental contaminant that is produced by human activities such as the production of dyes (Li et al., 2009), explosives, and pharmaceuticals (Gnanaprakasam and Selvaraju, 2014). It is used as a starting material in the production of latex, pesticides, and medicine, and is occasionally detected in the waste waters of military facilities and textile industries. Because of its extremely toxic, malignant, and genotoxic properties, even low concentrations of 2-NAN in water are harmful to aquatic and human life (Jimenez Jado et al., 2004).

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Various methods for the determination of aniline and 2-nitroaniline have been described. These methods include gas chromatography–mass spectrometry (GC–MS)(Singh et al., 2003), Electrosorption-enhanced solid-phase microextraction (EE-SPME) (Chai et al., 2007), high-performance liquid chromatography (HPLC) (Al-Kindy et al., 2013), and orthogonal signal correction–partial least squares (OSC-PLS) (Ghasemi and Niazi, 2005).

Derivative spectrophotometry (DS) plays an important role in multi-component analysis. The normal spectrum is differentiated by transforming the spectral curve into 1st or higher-order derivatives using a mathematical transformation (Rojas and Ojeda, 2009). Due to its superior ability to distinguish closely adjacent peaks and identify weak peaks obscured by sharp peaks, derivative spectrophotometry has been widely used to improve the signal and resolve overlapping peak-signals in comparison to direct absorption spectra (Ojeda and Rojas, 2013), removing spectral interferences brought on by scattering of light, and improving the capability to identify small spectral features. As a result, the mixture analysis's sensitivity and specificity are improved (Parmar and Sharma, 2016). The method of DS has been commonly used for the simultaneous determination of several organic compounds (Rasheed et al., 2017, Qader et al., 2019, Omer and Fakhre, 2020, Azeez and Fakhre, 2022). This study determines AN and 2-NAN in tap water samples using a derivative spectrophotometric technique.

2. Experimental

2.1 Instrument

For spectrophotometric measurements, a Shimadzu UV–V double-beam spectrophotometer (UV 1800, Japan) was used. It was connected to a computer to record zero-order spectra, and a computer running the UV Probe software was used to register the various orders (1st and 2nd).

2.2 Reagents

Stock Solutions of aniline (10000 ppm): Aniline was prepared by diluting 0.125 mL to 100 mL in a volumetric flask after distillation purification. Diluting the stock solution with ethanol yielded each working standard solution (Othman and Abdulla, 2020).

2-Nitroaniline solution (1000 µg/mL): 0.1 gram of the substance was dissolved in ethanol and diluted to 100 mL in a volumetric flask. Ethanol was used to dilute the stock solution to create each working standard solution (Janghel et al., 2005).

2.3 Analysis by Derivative UV Spectrophotometry

An effective derivative spectrophotometry technique has been applied to the simultaneous quantification of aniline and 2-nitroaniline in their mixtures. In this study, various derivative orders and measurement types were proposed, such as 1st and 2nd derivatives for the same purpose.

2.4 Recommended Procedures

2.4.1 Simultaneous Determination of AN and 2-NAN in Binary Mixture

2.4.1.1 First and Second Derivative Spectrophotometric Determination of AN and 2-NAN in Binary Mixture

From their respective working standard solutions, accurate aliquots of AN and 2-NAN were transferred to two series of 10 mL volumetric flasks, then filled with ethanol. The initial sequences contain AN at a constant concentration of 5.0 µg/ mL and 2-NAN at concentrations ranging from 1.0 -11.0 µg/ mL. The second sequence includes a constant concentration of 2–NAN 5.0 µg mL-1 and variable concentrations of AN 1.0 - 20.0 µg /mL. The 1st and 2nd DS of the solutions were measured between 216.41-288.08 nm.

2.4.1.2 Ratio Spectra Derivative Method (RSD) for Determination of AN and 2-NAN in Binary Mixture

The ratio spectra were calculated by dividing the absorption spectrum of the binary mixture solution of AN at various concentrations by the standard absorption spectra of 2-NAN (11.0µ g/mL). The interval of $\Delta\lambda$ = 10 nm was used to record and trace the 1st derivative of ratio spectra. For the quantification of AN in binary mixtures, the amplitude at 316.64 nm (1DD316.64) was selected. Similarly, the 2-NAN concentration in a binary mixture was determined using AN 2.0 µg/mL as a divisor and $\Delta\lambda$ = 10 nm. The amplitude at 372.40 nm (1DD372.40) was chosen to measure the amount of 2-NAN in a binary mixture.

3. Results and Discussion

AN's normal UV absorption spectra closely overlap with 2-NAN's spectrum. Fig. 1 presents the zero-order spectrums of AN, 2-NAN, and their mixtures using ethanol as a solvent.

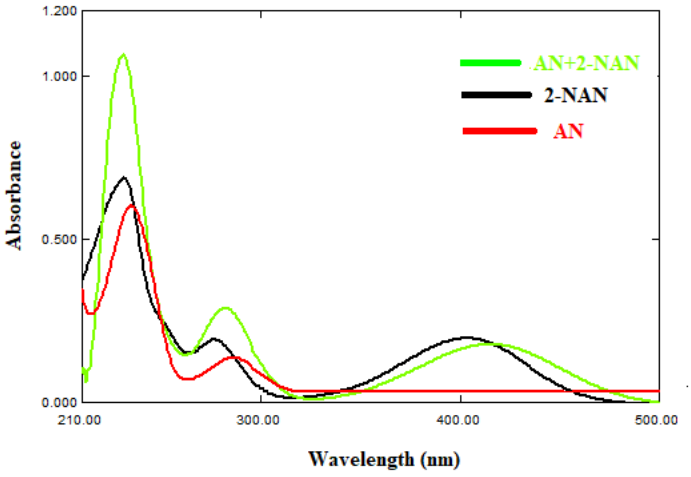


Figure 1: Zero-order spectrum of (5.0 µg/ mL) of 2-NAN, (5.0 µg /mL) of AN and their mixture

3.1 DS Techniques for Simultaneous Determination of AN and 2-NAN

The first order spectra of AN and 2-NAN, as well as their zero-crossing wavelengths, were inferred in Fig. (2A). The selected wavelength for the quantification of AN was 228.08 nm. The amplitude at this

wavelength is directly proportionate to AN concentration only (zero-crossing point of 2-NAN). On the other hand, the selected wavelength for the determination of 2-NAN was 234.39 nm, because at this wavelength 2-NAN peaks have amplitude values while the corresponding AN peaks read zero (zero-crossing point for AN). These wavelengths are optimal for simultaneously determining AN and 2-NAN in binary combinations. In addition, Fig. (2.B) displays the 2nd derivative absorption spectra of AN and 2-NAN. From this standpoint The zero-crossing method is used to determine AN in 2-NAN solutions. On the other hand, AN solution goes through zero absorption at wavelengths of 225.24 nm, while 2-NAN solution has absorption at wavelengths of 216.41 nm.

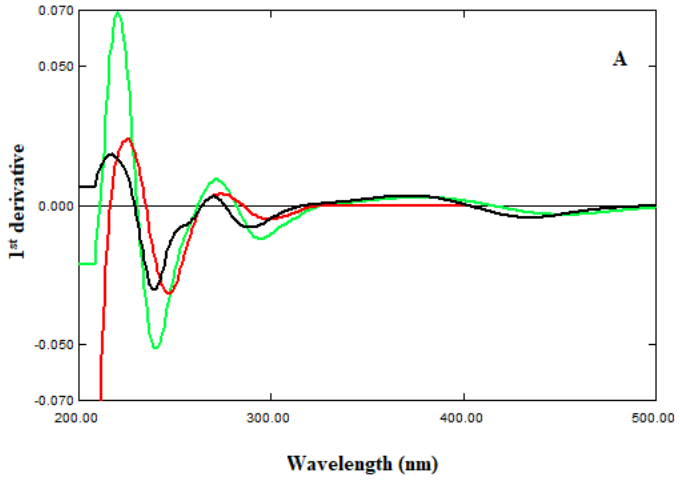


Figure 2 (A): 1st derivative spectra of ( ) 5.0 µg/ mL of AN, ( ) 5.0 µg/ mL of 2-NAN ( ) and their mixture

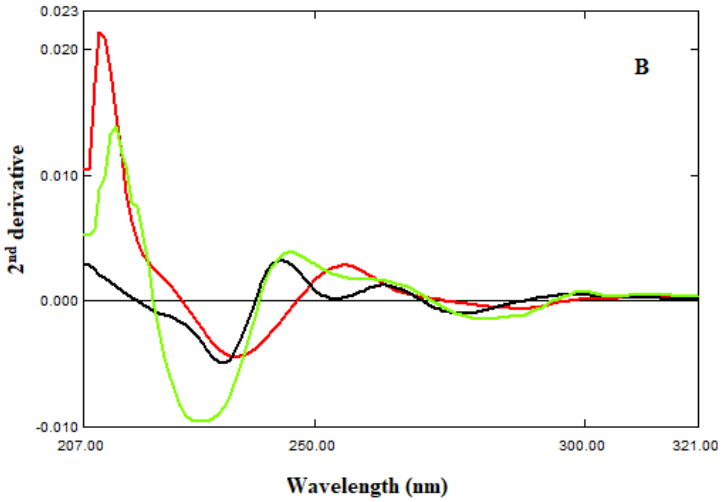


Figure 3 (B): Second -order derivative spectra of ( ) 5.0 µg/ mL of AN, ( ) 5.0 µg /mLof 2-NAN ( ) and their mixture

3.1.1 Calibration Curves and Statistical Data for Simultaneous Determination of AN with 2-NAN Using 1st and 2nd DS Technique

For the purpose of quantifying 2-NAN in the presence of AN, various mixture solutions of AN and 2-NAN were prepared so that the AN concentration remained constant at 5.0 µg/ mL while 2-NAN

concentrations varied. First and second derivative spectrum of the solutions were registered. For the quantification of AN in the presence of 2-NAN, various mixture solutions of AN and 2-NAN were prepared in which the concentration of 2-NAN was maintained at 5.0 µg mL-1 while the concentration of AN varied. The normal, 1st, and 2nd derivative spectra of the solutions are depicted in Figs. (3A), (3B), (4A), and (4B).

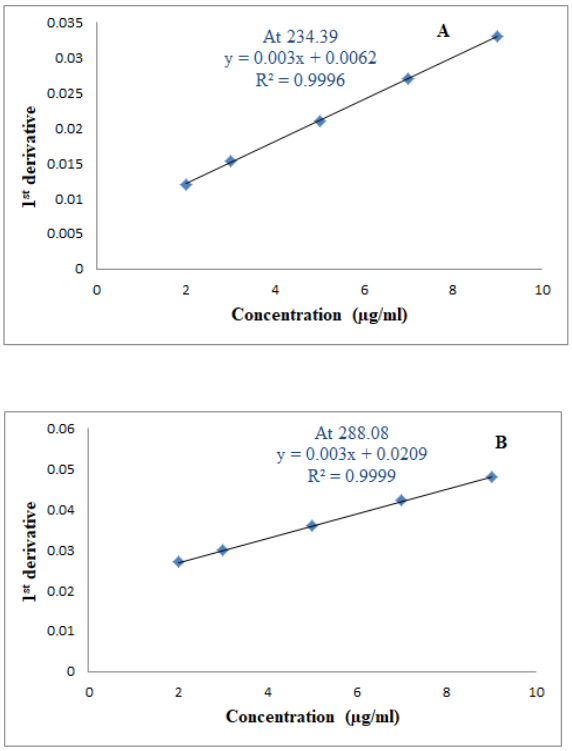
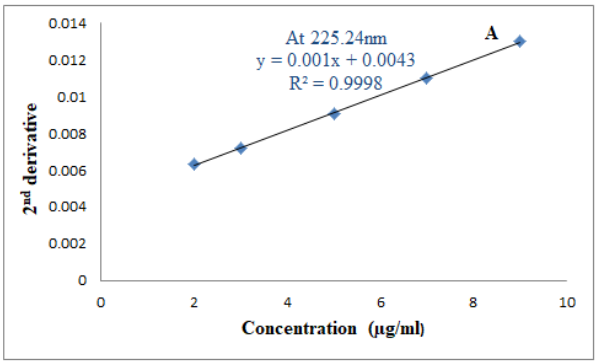


Figure 4: Calibration curves of first derivative spectrophotometric determination of (A) 2-NAN in the existence of (5.0 µg/ mL) AN, (B) AN in the presence (5µg/ mL) of 2-NAN, using zero crossing technique



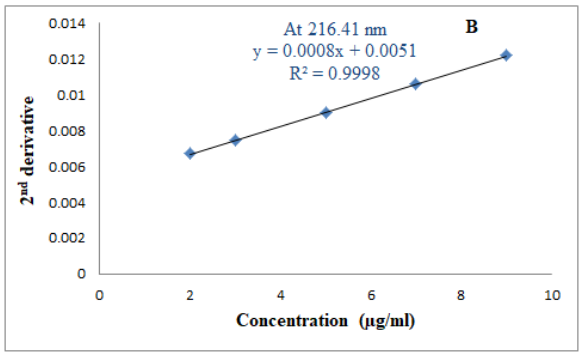
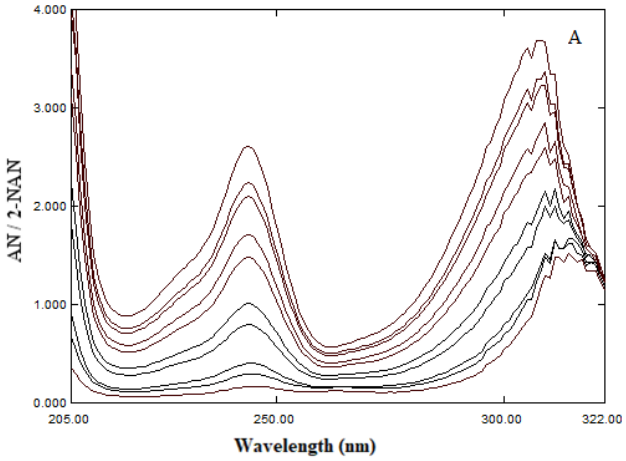


Figure 5: Calibration curves of second derivative spectrophotometric determination of (A) 2-NAN in the appearance of(5.0 µg /mL) AN, (B) AN in the presence (5µg/ mL) of 2-NAN, using zero crossing technique

3.2 Ratio Spectra Derivative Method for Determination of AN and 2-NAN in Binary Mixture

Absorption spectra of 1.0 - 20.0 µg/ mL AN solution were recorded between 205 and 360 nm. These spectra were divided by spectra of 2-NAN at 0.5–11.0 µg/ mL. Fig. (5A) shows the ratio spectra. Later, the first derivative of the solution in ethanol was recorded from RSD and traced with $\Delta\lambda$ = 10 nm, as shown in Fig. (5B). The AN concentration in a binary mixture was determined using the amplitude at 316.64 nm (1DD316.64). Fig. (6A) shows the RSD of 2-NAN obtained by dividing the spectra of 2-NAN at 0.5-11.0 µg/ mL in a binary mixture by the absorption spectrum of AN 2.0 µg/ mL. As shown in Fig.(6B), the 1st derivative was calculated from the ratio-spectrum and $\Delta\lambda$ =10 nm. The 2-NAN concentration in a binary mixture was determined using the 372.40 nm amplitude (1DD372.40). Figs. (7A) and (7B) show the simultaneous determination of AN and 2-NAN using RSD.



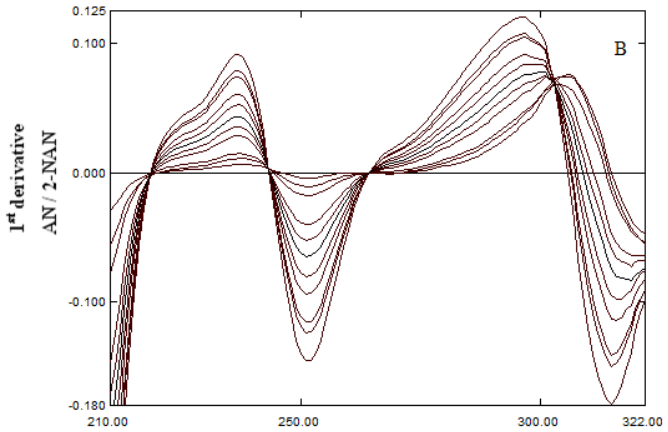


Figure 6: Ratio spectra (A) and 1st derivative (B) of AN (1.0-20.0 µg/ mL) when (11.0 µg/ mL) of 2-NAN used as divisor in ethanol (Δλ= 10nm)

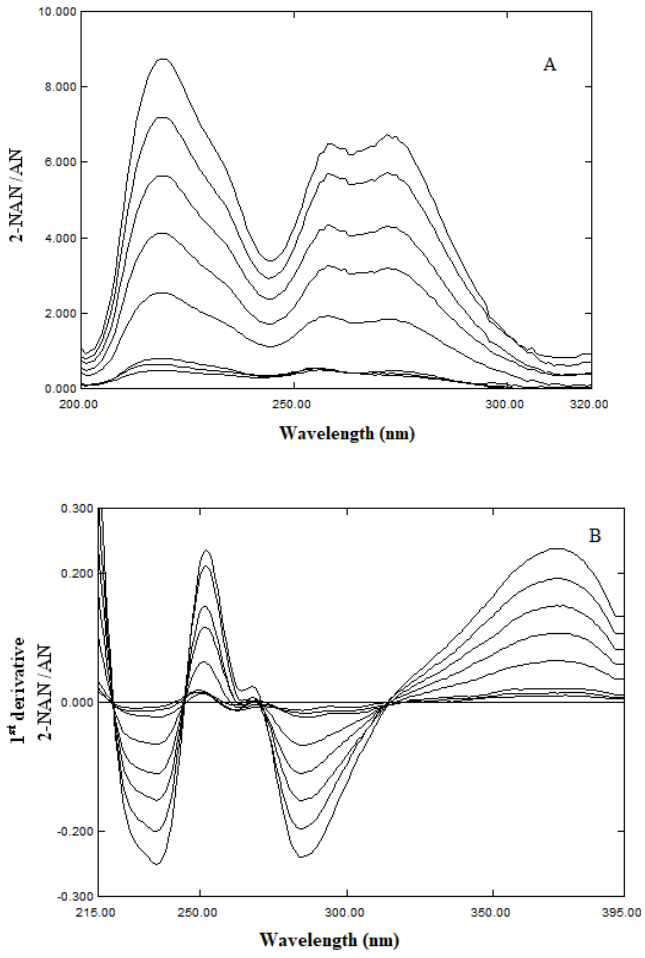
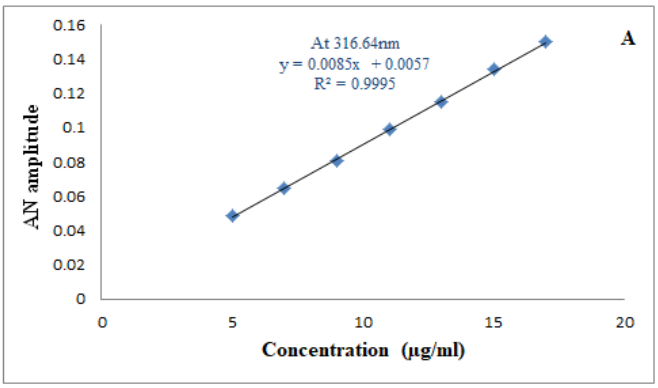


Figure 7: Ratio spectra (A) and 1st derivative (B) of 2-NAN (0.5-11.0 µg/ mL) when (2.0 µg /mL)of AN used as divisor in ethanol (Δλ= 10nm)



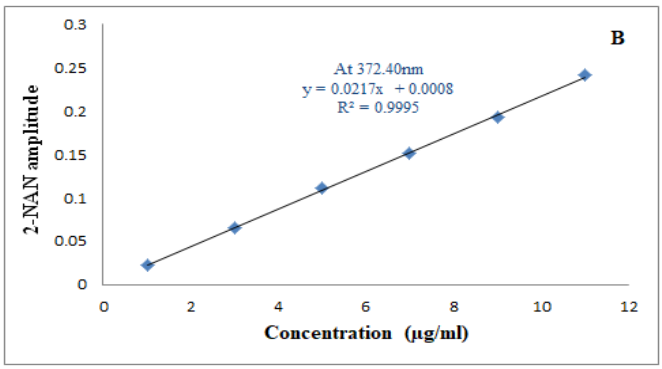


Figure 8: Calibration curves of (A) AN, (B) 2-NAN, using ratio spectra derivative method

3.3 Validation of the Methods

3.3.1 Calibration graph and Statistical Data

For the simultaneous estimation of binary mixtures of AN and 2-NAN using three different spectrophotometric methods, correlation coefficients, the linear range of the calibration curve, the limit of detection (LOD), and the limit of quantification (LOQ) were calculated. The results are shown in Table (1).

3.3.2 Accuracy and Precision

The 1st, 2ndand ratio spectra derivative spectrophotometric methods for simultaneous determination of AN with 2-NAN in binary mixtures under linearity were studied depending upon the values of the error percentage (Error %) and relative standard deviation percentage (RSD%) for three different standard concentrations based on five replicate measurements. The results are illustrated in Table (2).

3.3.3 Application of the Method

The proposed 1D, 2D zero-cross, and ratio spectra derivative techniques successfully determined AN and 2-NAN in tap water samples collected in the laboratory at the college of education using the standard addition method. Furthermore, recovery experiments were performed using the standard addition method, in which different amounts of standard AN and 2-NAN solutions were added to 10 ml of tap water samples, as demonstrated in Table (3).

Table 1: The statistical parameters for determining AN and 2-NAN using proposed methods

Method of analysis	Compounds	Wavelength (nm)	Linear range (µg/mL)	Regression Equation	r^2	Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	LOD (µg/mL)	LOQ (µg/mL)
Binary mixture of AN with 2-NAN by 1st derivative	AN	288.08	1.0-20	y= 0.003x+0.0209	0.9999	2.8149×10^2	0.18	0.55
	2-NAN	234.39	1.0-11	y= 1.3799x-0.615	0.9997	4.1071×10^2	0.14	0.25
Binary mixture of AN with 2-NAN by 2nd derivative	AN	216.41	1.0-20	y= 0.0008x+0.0051	0.9998	7.279×10^2	0.19	0.57
	2-NAN	225.24	1.0-11	y= 0.001x+0.0043	0.9991	1.3216×10^2	0.09	0.27
RSD	AN	^{1}D316.64	1.0-20.0	y= 0.0085x+0.0057	0.9995	7.1244×10^2	0.34	0.36
	2-NAN	^{1}D372.40	0.5-11.0	y= 0.0217x+0.0008	0.9995	1.3331×10^2	0.29	0.39

Table 2: The precision and accuracy for determining AN and 2-NAN simultaneously in a binary mixture

Method of analysis	Compounds	Wavelength (nm)	Concentration (µg/mL)	RSD%	Error%
Binary mixture of AN&2-NAN by 1st derivative	AN	288.08	2.0	4.7	+1.67
			5.0	4.09	+0.67
			9.0	3.01	+0.74
	2-NAN	234.39	2.0	2.24	-3.33
			5.0	4.55	-1.33
			9.0	4.83	-0.74
Binary mixture of AN&2-NAN by 2nd derivative	AN	216.41	3.0	3.68	0.0
			7.0	0.99	-1.79
			9.0	3.39	-1.39
	2-NAN	225.24	3.0	2.18	-3.33
			7.0	1.37	-4.29
			9.0	4.68	-3.33
RSD	AN	274.05	1.0	1.04	-3.13
			9.0	0.49	+1.53
			20.0	2.25	-0.11
		300.60	2.0	2.34	-1.45
			9.0	0.47	+0.81
			20.0	0.37	-1.29
RSD	2-NAN	220	3.0	0.46	-1.01
			7.0	0.19	-0.92
			13.0	0.17	+0.09

Table 3: Simultaneous determination of AN and 2-NAN in tap water samples

Method of analysis	Compounds	Wavelength (nm)	Add amount (µg/mL)	Found amount (µg/mL)	Recovery %
Binary mixture of AN with 2-NAN using 1st derivative	AN	288.08	2.0	2.03	101.66
	2-NAN	234.39	3.0	3.1	102.22
Binary mixture of NA with 2-NAN using 2nd derivative	AN	216.41	5.0	4.9	97.5
	2-NAN	225.24	3.0	2.9	96.67
Binary mixture of AN with 2-NAN using	AN	316.64	5.0	5.0	101.9
			11.0	10.9	99.8

Ratio spectra derivative			17.0	16.9	99.9
	2-NAN	372.40	1.0	0.98	97.7
			5.0	5.1	101.6
			11.0	11.1	100.6

4. Statistical Analysis

GraphPad Prism 9.0 was used to perform the statistical analysis. The Anova test was used to compare the AN and 2-NAN's first, second, and ratio derivative spectrophotometry spectra. As shown in Table (4), a *p* value < 0.05 was considered statistically significant.

Table 4: Statistical analysis results of AN and 2-NAN

Compounds		Sum of squares	Degree of freedom	Mean squares	F	P value
Aniline	Between groups	0.6303	2	0.3151	0.011	0.98
	Within groups	556.4	18	30.91		
	Total	557.1	20			
2-Nitroaniline	Between groups	0.1071	2	0.05353	0.029	0.97
	Within groups	100.3	12	8.359		
	Total	100.4	14			

5. Conclusion

The zero-crossing and ratio spectral methods were successfully used to determine aniline and 2-nitroaniline in tap water samples. These methods successfully resolve a binary mixture without complex or expensive equipment. Moreover, the derivative spectra of binary mixtures, including AN and 2-NAN, were determined. The results had good linearity. The high values of correlation coefficients indicate the good linearity of all calibration curves to derivative measurements, and the application seems to produce good recovery results.

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