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CHEMOMETRIC SMART APPROACHES USING ARTIFICIAL NEURAL NETWORKS AND CONTINUOUS WAVELET TRANSFORM FOR SIMULTANEOUS QUANTITATIVE ANALYSIS OF CIPROFLOXACIN-ORNIDAZOLE TABLETS

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ABSTRACT. New chemometric smart approaches, Artificial Neural Network (ANN) and Continuous Wavelet Transform (CWT), based on UV spectrophotometric data, were proposed for the simultaneous quantitative analysis of ciprofloxacin and ornidazole in tablets. Both methods enabled the study of the two-component mixtures containing these drugs without requiring a pre-separation process. The ANN calibration model was developed by establishing a relationship between the absorbance measurement matrix and the calibration set, which was constructed using a full factorial design methodology. To quantify ciprofloxacin and ornidazole, Symlets8 continuous wavelet transform (sym8-CWT) exhibited to be a suitable tool for transforming the UV spectra during the calibration and prediction stages. Both chemometric methods were applied within the linear working range of 3–24 µg/mL for ciprofloxacin (CIP) and 6–32 µg/mL for ornidazole (ORN). The validity of the proposed ANN and sym8-CWT approaches was confirmed through the analysis of independent test samples, as well as intra-day, inter-day, and standard addition experiments. The ANN method provided impressive recovery rates of 99.9% for CIP and 100.1% for ORN. Similarly, the sym8-CWT method achieved recovery rates of 98.5% for CIP and 101.5% for ORN. Both ANN and sym8-CWT approaches were successfully applied to the real sample analysis of CIP-ORN tablets, demonstrating precise and accurate results at a low cost and with minimal sample preparation.

Keywords: *UV-Spectrophotometry, Artificial Neural Network, Continuous Wavelet Transform, Ciprofloxacin, Ornidazole*

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INTRODUCTION

Ciprofloxacin, chemically known as 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid (Figure 1), is an antibiotic effective against a wide range of bacterial infections. It exhibits activity against both Gram-positive (Gram +) bacteria, such as Streptococcus pneumoniae, and Gram-negative (Gram -) bacteria, such as Helicobacter pylori. Ciprofloxacin is commonly prescribed for urinary tract infections, certain gastrointestinal infections, gynecological infections, sexually transmitted diseases, skin infections, and upper and lower respiratory tract infections, including sinusitis, pneumonia, and bronchitis [1].

Ornidazole, chemically named 1-Chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol, is an antibiotic primarily used to treat protozoan infections. Its antimicrobial spectrum is similar to that of metronidazole, but it is better tolerated. Initially, ornidazole was introduced to treat trichomoniasis, a condition caused by protozoan and anaerobic bacterial infections [2]. It has also been found effective in managing Crohn's disease [3]. Additionally, ornidazole demonstrates activity against coliform bacteria in vivo, despite in vitro resistance [4]. It is suggested that the drug remains active against aerobic Gram-negative microorganisms in the presence of anaerobic bacteria [5].

In previous studies, various analytical methods, including high-performance liquid chromatography (HPLC) [1–4, 6–9] and spectroscopy [5–8, 10–13], have been documented for analyzing the combination of ciprofloxacin and ornidazole in tablets. Additionally, the estimation of these substances in spiked serum has been conducted using thin-layer chromatography (TLC) [14] and HPLC [15].

While widely used, Chromatographic methods come with notable drawbacks, including the need for sophisticated instrumentation, time-consuming procedures, high costs, and a considerable environmental impact [16–18]. In contrast, spectrophotometry offers a straightforward, cost-effective alternative that does not require complex steps or processes. However, one of the key challenges in spectrophotometric analysis is the spectral overlap of analytes, where the absorption spectra of different components in a mixture may interfere with one another, complicating accurate analysis.

This issue of spectral data overlap can be effectively addressed by integrating chemometric methods with spectrophotometric data. Unlike chromatographic techniques, this approach eliminates the need for tedious sample pretreatment [19, 20]. Spectrophotometry, as an analytical technique, measures the absorption or transmission of light by a sample as a function of wavelength, providing a simple and economical method for analysis. When

combined with advanced chemometric tools, it becomes a powerful solution for resolving spectral overlaps, offering reliable and practical results without extensive sample preparation [19-20].

Additionally, the combined use of spectrophotometric data with chemometric methods aligns with the principles of green analytical chemistry, enhancing the “greenness” of the analytical process. This synergy reduces resource consumption through miniaturization, minimizes using bio-accumulative or non-green reagents, and decreases waste production. Tools like the Analytical Eco-Scale and the National Environmental Methods Index (NEMI) are commonly employed to evaluate the environmental sustainability of such methods, grounded in the twelve principles of green chemistry [21].

Although liquid chromatographic techniques are often applied to quantify overlapping spectra of drugs with similar chemical structures, they are less favored in green analytical practices due to their higher environmental impact. The combination of spectrophotometric techniques and chemometric methods offers an efficient, eco-friendly alternative for accurate and sustainable pharmaceutical analysis.

In recent years, using two or more active substances rather than a single active ingredient in pharmaceutical formulations has become increasingly common. This approach aims to achieve enhanced therapeutic effects or prevent the progression of diseases by targeting multiple pathways or mechanisms. However, the inclusion of multiple active compounds in a formulation introduces significant challenges in terms of quality control and routine analytical procedures. As previously mentioned, a significant challenge is the complexity caused by overlapping spectral bands of analytes within the same spectral region, making it impossible to analyze multicomponent mixtures using direct absorbance measurement techniques.

To overcome this challenge, advanced chemometric techniques such as Artificial Neural Networks (ANN) and Continuous Wavelet Transform (CWT) have proven to be highly effective tools. By utilizing ANN models and CWT signal processing on UV absorbance measurement data, accurate and reliable quantitative resolution of complex mixtures containing two or more analytes can be achieved. These methods provide a robust and innovative solution to the limitations of conventional analytical techniques, enabling more efficient, precise, and reliable analysis in pharmaceutical research and quality control applications.

Artificial Neural Network (ANN) and Continuous Wavelet Transform (CWT) methods are among the most significant numerical and graphical tools employed in academic and pharmaceutical sectors to address complex analytical problems, respectively, without the need for a preliminary separation procedure. Continuous Wavelet Transform (CWT) is a well-known method in

the field of chemometrics [22-24]. It employs wavelet functions to analyze spectral reflections at various scales, breaking down the spectral signal into a series of wavelet coefficients. CWT's most significant advantage is its ability to simultaneously detect the phase and instantaneous frequency of a nonstationary signal, which is achieved through time-frequency analysis.

Although liquid chromatographic techniques effectively resolve overlapping spectra of similar drugs, their high resource demands, and environmental impact make them less favorable. Chemometric methods, including Continuous Wavelet Transform (CWT) and Artificial Neural Networks (ANN), overcome these limitations by improving signal-to-noise ratios and minimizing spectral interference. Despite challenges in determining zero points and selecting optimal wavelet families, recent studies highlight the successful application of CWT, ANN, and UV-Vis spectrophotometry for the simultaneous quantification of ciprofloxacin (CIP) and ornidazole (ORN) in synthetic mixtures and commercial formulations.

This study aimed to improve advanced methods, Continuous Wavelet Transforms (CWTs), and Artificial Neural Networks (ANN) using the UV absorbance measurements for the simultaneous quantitative estimation of ciprofloxacin (CIP) and ornidazole (ORN) in a tablet dosage form. Among the various wavelet functions tested, the symlets8 family was identified as the most suitable for the determination of these drugs. Calibration graphs for CIP and ORN were established within their respective working ranges of 3–24 µg/mL and 6–32 µg/mL, yielding accurate and reliable results. In the architect of the ANN model, 273 input neurons, five hidden neurons, and two output neurons were used for the ANN chemometric calibration using full absorbance measurement and a concentration set of 17 mixtures containing CIP and ORN drugs. After validation of the proposed chemometric approaches, the methods were applied to quantify the relevant substances (CIP and ORN) in commercial tablets.

RESULTS AND DISCUSSION

In analytical practice, one of the key challenges when applying Continuous Wavelet Transform (CWT) for resolving overlapping UV spectral bands is identifying the most suitable wavelet family that provides optimal quantification of the relevant drugs in their mixture. In the present study, various wavelet families with distinct scaling factors were systematically tested for their ability to resolve the overlapping spectral bands of ciprofloxacin (CIP) and ornidazole (ORN). Among the tested families, the sym8-CWT wavelet family emerged as the most effective tool, delivering precise, accurate, and reliable results for the simultaneous quantitative estimation of CIP and ORN

in tablet formulations. The enhanced application of the sym8-CWT approach, in conjunction with the Artificial Neural Network (ANN) model, is discussed in detail below.

As previously outlined, calibration solutions of CIP and ORN were prepared within their respective working ranges of 3.0–24.0 µg/mL for CIP and 6.0–32.0 µg/mL for ORN. The UV spectra of the calibration standards, test samples (binary mixtures), and commercial tablet formulations were recorded across a wavelength range of 220–400 nm. The spectral overlap of CIP and ORN was clearly illustrated in Figure 1. As observed, traditional spectrophotometric methods are insufficient for the simultaneous quantitative analysis of CIP and ORN due to the pronounced overlap of their UV absorption spectra within the same spectral region. This spectral interference poses a significant challenge in distinguishing between the two analytes in complex tablet matrices.

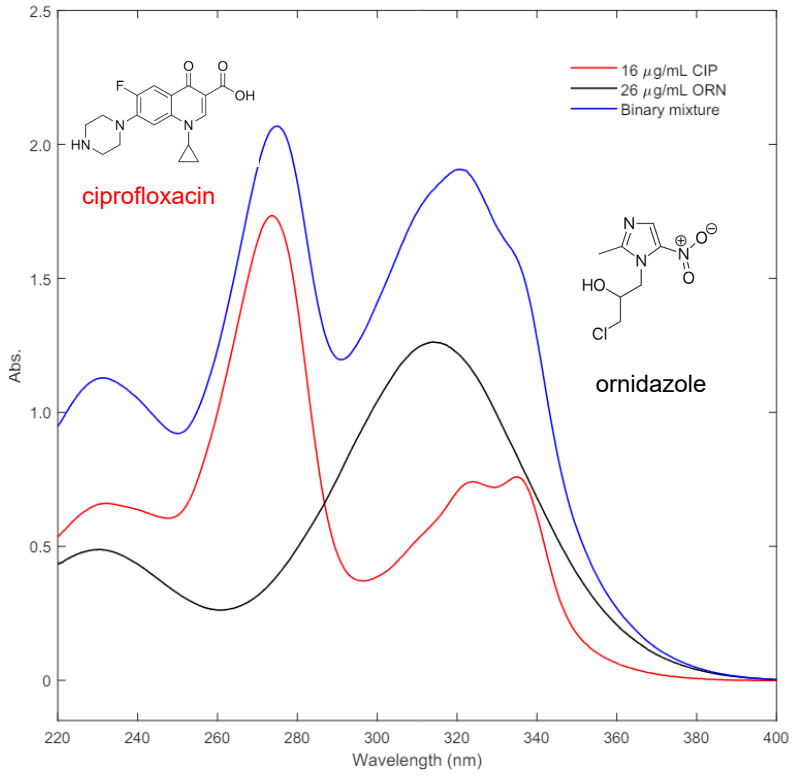

Figure 1. Chemical structure of CIP and ORN, UV spectrum of CIP, ORN and mixture of CIP and ORN

To overcome this limitation, we explored novel signal processing techniques based on wavelets, which have been proven effective for resolving mixtures of compounds without the need for complex sample separation steps. The preliminary results of these tests revealed that the sym8-CWT wavelet family was the most suitable for transforming the UV spectral data vectors of both the calibration and validation samples. This transformation enabled accurate and reliable quantitative analysis of the CIP-ORN tablets, highlighting the robustness of the sym8-CWT approach in complex pharmaceutical formulations.

Application of ANN and CWT Methods

As explained above, the calibration samples containing CIP and ORN in the concentration range of 3.0-24.0 µg/mL for CIP and 6.0-32.0 µg/mL for ORN in base media and methanol were individually prepared by using standard stock solutions of the analyzed compounds. The absorption spectra of the analyzed samples were recorded between 220.0-400.0 nm. The recorded absorbance data vectors were transferred into Microsoft Excel. This Excel file for the wavelet analysis of the data was saved on the computer. Figure 1 indicates the chemical structures of CIP and ORN, the UV spectrum of CIP (16 µg/mL), ORN (28 µg/mL), and a mixture of CIP (16 µg/mL) and ORN (28 µg/mL). Figure 2a shows the UV absorption spectra of the calibration samples of CIP and ORN in their working concentration range. Some continuous wavelet families at different scale parameters (a) were applied to process the UV absorbance data vectors. From the wavelet analyses, sym8-CWT was identified to get the optimal signal transformation of the original UV spectra for more precise and accurate assay results. The CWT spectra of CIP and ORN, obtained by applying the CWT signal processing methods to the absorbance data vectors, are indicated in Figure 2b. From these CWT spectra in Figure 2, it was observed that three different CWT approaches became very suitable for resolving overlapping UV spectra for the analysis of CIP-ORN tablets.

For the quantitative estimation of the related substances, the calibration curves were obtained by regression of the concentration on the sym8-CWT amplitude at 320 nm and 341 nm for CIP and ORN, respectively (Figure 2b). The statistical results related to the linear regression analyses for the analyzed active compounds are listed in Table 1. CIP and ORN were determined by means of the computed calibration curves.

In the application of the ANN model, input 273 neurons, hidden 5 neurons, and output 2 neurons were used as a methodological architect. A concentration set containing 17 mixtures of CIP and ORN was prepared using the full factorial design with two factors and four levels (Table 2.).

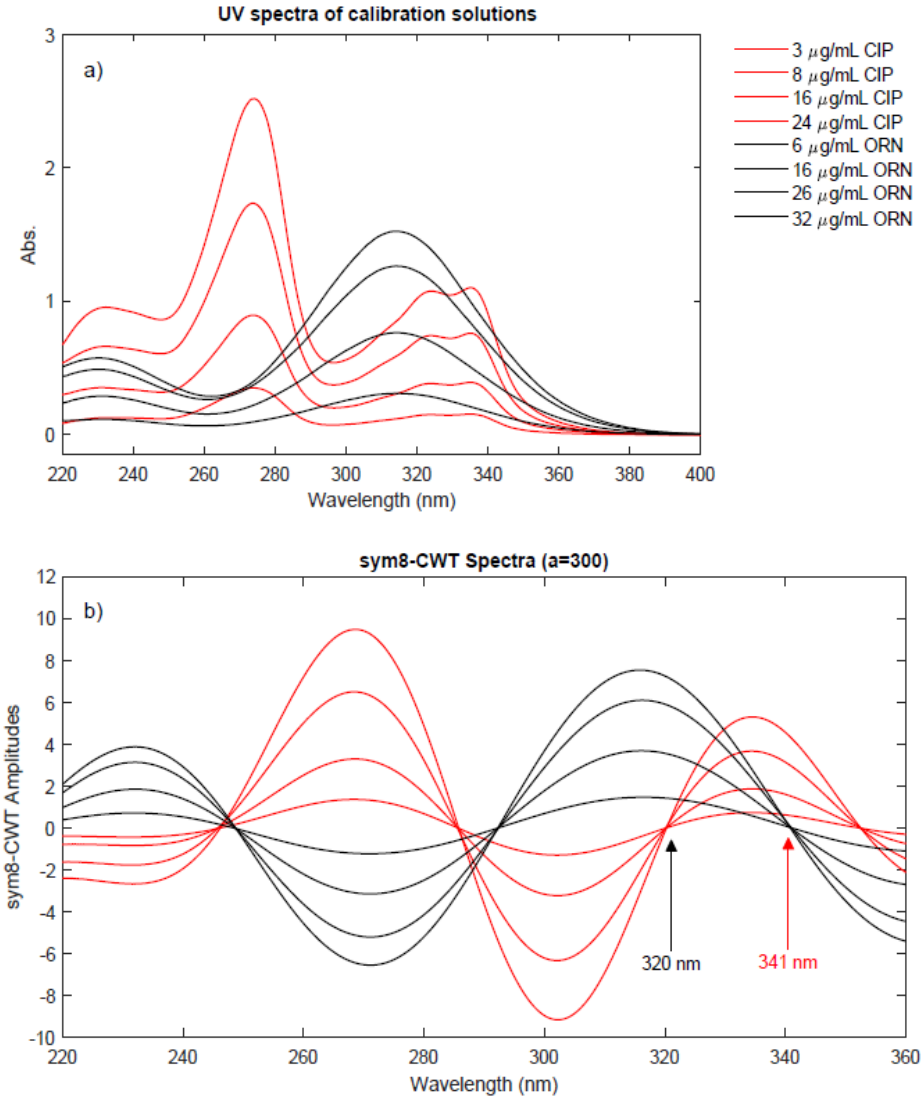

Figure 2. Zero order absorption spectra (a), sym8-CWT method (b), of 3-24 µg/mL CIP (—) and 6-32 µg/mL ORN (—) mixtures using methanol as blank.

Table 1. Calibration results obtained by the proposed sym8-CWT method for the analysis of CIP and ORN in mixture samples

	CIP	ORN
m	0.1615	0.2195
n	0.0760	0.0424
r	0.9998	0.9998
SD(m)	2.35×10^{-3}	3.12×10^{-3}
SD(n)	3.53×10^{-2}	6.97×10^{-2}
SD(r)	1.03×10^{-2}	1.01×10^{-2}
LOD	0.66	0.95
LOQ	2.19	3.17

m=slope; n=intercept; r=correlation coefficient; SD (m)=standard deviation of slope; SD (n)=standard deviation of intercept; SD (r)=Standard deviation of correlation coefficient; LOD=detection limit (µg/mL); LOQ=quantitation limit (µg/mL)

In building the ANN calibration procedure, the performance of the method is illustrated in Figure 3. The build ANN model was applied to the quantification of the related drugs in analyzed samples.

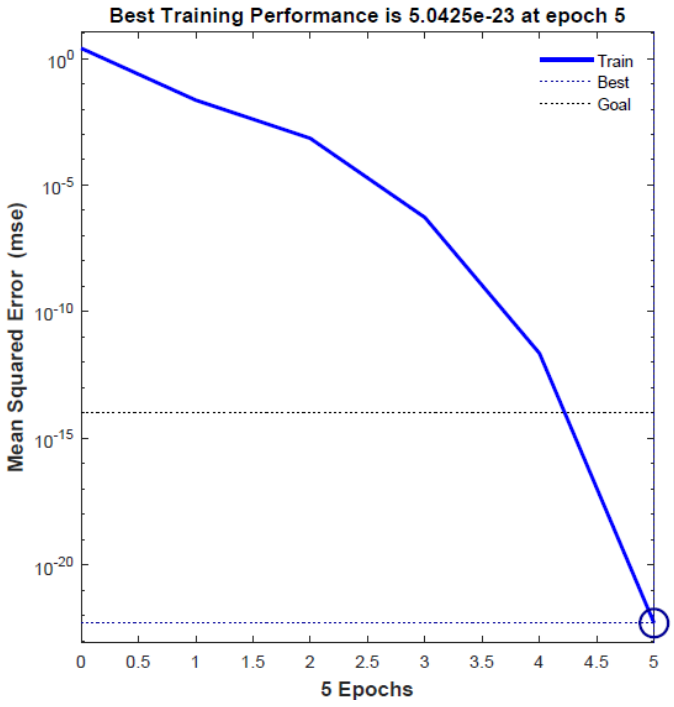

Figure 3. Performance of ANN method

Analytical validity of the applied methods

In this research, the analytical validity of the applied methods, ANN and sym8-CWT, was assessed based on several factors, including range, linearity, accuracy, precision, selectivity, limit of detection (LOD), and limit of quantitation (LOQ) for the analysis of CIP and ORN in tablets. The results showed that the concentration range for both compounds was linear, with a range of 3.0–24.0 µg/mL for CIP and 6.0–32.0 µg/mL for ORN. Higher correlation coefficients were obtained for the calibration curves of CIP and ORN using sym8-CWT, as shown in Table 1. In the validation process, LOD and LOQ were determined using the standard deviation and slope values from the calibration curves, with the corresponding results presented in Table 1.

Table 2. Full factorial design of the analysis

Sample Number	CIP µg/mL	ORN µg/mL
K2	8	6
K3	16	6
K4	24	6
K5	3	16
K6	8	16
K7	16	16
K8	24	16
K9	3	26
K10	8	26
K11	16	26
K12	24	26
K13	3	32
K14	8	32
K15	16	32
K16	24	32
K17	18	18

Several binary mixtures with different concentration levels of the active compounds were analyzed to evaluate the accuracy and precision of the proposed methods, as described in the recovery and intra-day and inter-day studies. Percent mean recoveries and relative standard deviations were calculated. The assay results, obtained using the signal processing methods, are presented in Tables 3 and 4. The experiments showed that the proposed wavelet and derivative methods provided satisfactory accuracy and precision without requiring a preliminary separation step.

Table 3. Recovery results of CIP and ORN in laboratory prepared mixtures by the ANN and CWT methods

			ANN				sym8-CWT			
Sample	Added (μg/mL)		Found (μg/mL)		Recovery (%)		Found (μg/mL)		Recovery (%)	
Code	CIP	ORN	CIP	ORN	CIP	ORN	CIP	ORN	CIP	ORN
M1	3	18	2.97	17.62	99	97.9	3	18.29	100.1	101.6
M2	12	18	12.26	18.28	102.2	101.6	12.03	18.81	100.2	104.5
M3	24	18	23.31	17.95	97.1	99.7	23.29	18.37	97	102
M4	18	6	18.25	5.94	101.4	98.9	17.46	6.11	97	101.8
M5	18	20	17.73	20.23	98.5	101.2	17.7	19.72	98.3	98.6
M6	18	32	18.15	32.39	100.8	101.2	17.66	32.1	98.1	100.3
				Mean	99.9	100.1		Mean	98.5	101.5
				SD	1.94	1.48		SD	1.86	1.89
				RSD	1.94	1.47		RSD	1.85	1.9

$\bar{X}$: Mean
SD: Standard deviation
RSD: Relative standard deviation

To assess the method's precision and accuracy, calculations for percent mean recovery and relative standard deviation (%) for the test samples, prepared as described in the "Preparation of Validation Samples" section, are shown in Table 3. The optimized ANN and CWT methods performed well in analyzing independent test samples containing CIP and ORN at various concentration levels, as indicated in the table.

To evaluate the precision and accuracy of the intra-day and inter-day analysis using the optimized ANN and sym8-CWT methods, samples were prepared at three different concentration levels: 6.0, 12.0, and 18.0 μg/mL for CIP and 8.0, 16.0, and 24.0 μg/mL for ORN. These samples were analyzed three times within the same day (intra-day) and over three consecutive days (inter-day). This process was repeated three times for each concentration level. The results of the intra-day and inter-day assays, obtained using the optimized ANN and CWT methods, are shown in Table 4. As indicated in the table, the recovery results were satisfactory, demonstrating high precision and accuracy. The table also presents the relative standard deviations and relative standard errors for both drugs.

To assess the specificity and selectivity of the method and determine whether tablet excipients influence the analysis of the target drugs, a recovery study was performed using standard addition (spiked) samples. As outlined in the "Preparation of Validation Samples" section, these samples were prepared by adding known quantities of the investigated compounds into a portion of the tablet solution. The total amounts of the analytes in the spiked samples were determined by comparing the measured peak areas

Table 4. Intra-day and inter-day results of CIP and ORN in mixture samples by the proposed ANN and CWT method

	ANN				Sym8-CWT			
	Added (µg/mL)		Found (µg/mL)		Added (µg/mL)		Found (µg/mL)	
	CIP	ORN	CIP	ORN	CIP	ORN	CIP	ORN
Inter-day	6	8	6.23	8.09	6	8	5.99	8.13
	12	16	12.7	16.15	12	16	12.14	16.46
	18	24	18.11	24.71	18	24	18.18	24.22
Intra-day	6	8	6.1	8.19	6	8	5.86	7.95
	12	16	12.13	16.27	12	16	12.04	16.53
	18	24	17.76	24.23	18	24	17.66	23.78
			Recovery (%)				Recovery (%)	
Inter-day			103.8	101.1			99.9	101.6
			105.8	100.9			101.2	102.9
			100.6	102.9			101.0	100.9
Intra-day			101.7	102.3			97.7	99.4
			101.1	101.7			100.3	103.3
			98.6	100.9			98.10	99.10
			RSD (%)				RSD (%)	
Inter-day			0.15	1.37			1.29	1.10
			0.86	0.61			1.45	0.82
			1.32	0.66			0.84	0.58
Intra-day			0.49	2.1			1.17	0.54
			0.51	0.32			1.30	1.59
			0.4	0.59			1.05	0.52
			RSE (%)				RSE (%)	
Inter-day			3.80	1.12			-0.10	1.61
			5.81	0.92			1.15	2.86
			0.63	2.94			1.00	0.92
Intra-day			1.70	2.35			-2.34	-0.59
			1.12	1.69			0.33	3.32
			-1.35	0.94			-1.91	-0.91

RSD: Relative standard deviation
RSE: Relative standard error

against the calibration curves. Subsequently, the amounts of the individual drugs were calculated by subtracting the drug content in the tablet solution from the total amount found in the spiked sample. Recovery and relative standard deviation (RSD) were then calculated using the formulas: Recovery (%) = (found amount/added amount) × 100 and RSD (%) = (SD/mean) × 100. The results for added recovery and standard deviations are provided in Table 5. The analysis of the spiked samples demonstrated no interference from excipients, confirming that the spectrophotometric data were suitable for the analysis and that each spectrum contained only the target drug.

Table 5. Standard addition results of CIP and ORN in mixture samples by the proposed CWT methods

	ANN			
	Added (µg/mL)		Found (µg/mL)	
	CIP	ORN	CIP	ORN
Formulation	5	6	4.95	5.98
Formulation	10	12	10.68	11.96
Formulation	15	18	15.11	17.76
			Recovery (%)	
			99	99.6
			106.8	99.7
			100.8	98.7
			RSD (%)	
			1.21	0.09
			0.1	0.05
			0.06	0.16
	Sym8-CWT			
	Added (µg/mL)		Found (µg/mL)	
	CIP	ORN	CIP	ORN
Formulation	5	6	4.96	6.12
Formulation	10	12	9.96	12.18
Formulation	15	18	14.73	18.3
			Recovery (%)	
			99.3	102
			99.6	101.5
			98.2	101.7
			RSD (%)	
			0.03	0.02
			0.18	0.11
			0.11	0.1

RSD: Relative standard deviation

Analysis of Commercial Tablets

In this study, the quantification of CIP and ORN in commercial tablets was accurately carried out using the innovative ANN and sym8-CWT methodologies. The results of these analyses, obtained through the proposed analytical approaches, are presented in Table 6. The UV spectra of the commercial tablets were recorded within the wavelength range of 220-400 nm. Subsequently, these spectra were processed through advanced signal processing techniques, facilitating the precise and reliable determination of CIP and ORN concentrations in the tablets, as shown in Figure 1. A strong correlation

between the observed results underscores the robustness and applicability of the proposed methods, demonstrating their efficacy in the simultaneous quantification of CIP and ORN in pharmaceutical tablets.

Table 6. Determination of CIP and ORN in ORCIPOL ® tablets by the proposed ANN and CWT methods

ANN			sym8-CWT		
	mg/tablet			mg/tablet	
Experiment No.	CIP	ORN	Experiment No.	CIP	ORN
1	16.1	15.5	1	16.2	15.7
2	15.5	16.1	2	16	16
3	15.8	16	3	15.9	15.9
4	15.7	16	4	15.9	15.8
5	15.9	16.2	5	16.3	15.9
6	16.3	16.3	6	16.2	16.6
7	15.9	16.2	7	16.2	16.1
8	15.7	15.9	8	15.7	16.1
9	15.8	16.2	9	15.9	16.4
10	15.8	15.8	10	16.2	16.1
Mean	15.9	16	Mean	16	16.1
SD	0.24	0.24	SD	0.2	0.25
RSD	1.5	1.53	RSD	1.24	1.53

Label claim is 16 mg CIP and ORN in a tablet.
SD: standard deviation; RSD: Relative standard deviation.

CONCLUSIONS

In this study, we developed and validated the effectiveness of two distinct methods, Artificial Neural Networks (ANN) and Continuous Wavelet Transform (CWT), for the simultaneous quantitative analysis of binary mixtures of CIP and ORN. Both approaches were applied independently, and the results demonstrated that they provided comparable and reliable outcomes for the analysis of CIP and ORN in tablet formulations without requiring chemical pre-treatment or preliminary separation steps.

The ANN model and the sym8-CWT signal processing approach both proved to be highly effective in overcoming the challenge of spectral overlap, a common issue in spectrophotometric analysis. These methods showed excellent precision and accuracy, supporting their potential for routine analysis and quality control in pharmaceutical settings.

By using both ANN and CWT methods, we were able to achieve simultaneous and quantitative resolution of CIP and ORN in a commercial pharmaceutical formulation, further validating the applicability of these methods. The findings suggest that both approaches offer versatile solutions for spectrophotometric analysis in the presence of overlapping spectra, making them suitable alternatives to traditional separation techniques in pharmaceutical quality control.

EXPERIMENTAL SECTION

Apparat and software

In this study, a Shimadzu UV-2550 series double-beam UV-Vis spectrophotometer was employed to acquire UV absorption spectra of the analyzed samples. The system was interfaced with a computer running Shimadzu UV Probe 2.32 software, which facilitated data collection and spectral analysis.

The development and implementation of the Artificial Neural Network (ANN) and Continuous Wavelet Transform (CWT) approaches were carried out using the Wavelet Toolbox in MATLAB software. For statistical evaluation and data processing, Microsoft Excel software was used, ensuring a robust and reliable analysis workflow.

Chemicals and Material

In this study, the CIP and ORN standards were obtained from the reputable World Medicine Pharmaceuticals Company in Turkey, and the commercial product ORCIPOL tablet was utilized. Methanol, used as the solvent for standard and validation sample solutions, was procured from Fisher Scientific (UK), along with high-purity distilled water.

All sample solutions were freshly prepared daily at room temperature, ensuring consistency and reliability in the analysis. Methanol (Fisher Scientific (UK), HCl (Merck, Darmstadt-Germany) were used as the solvent for all solutions throughout the study. Laboratory-prepared mixtures of CIP and ORN were employed for pharmaceutical validation, serving as essential components in the analytical preparations.

In the analysis of the commercial real sample, the commercial ORCIPOL® tablet, manufactured by World Medicine Pharmaceuticals in Istanbul, Turkey, was selected as the real sample for analysis. Each tablet contains 554.92 mg (equivalent to 500 mg ciprofloxacin, CIP) of ciprofloxacin hydrochloride and 500 mg of ornidazole (ORN), and the label of excipient

content is sodium starch glycolate 141.44 mg, croscarmellose sodium 50 mg, sunset yellow FCF for film coating, and tartrazine for film coating. To analyze this formulation, two advanced methods, Artificial Neural Network (ANN) and Symlet 8 Continuous Wavelet Transform (sym8-CWT) were employed.

Preparation of Standard Stock Solutions and Calibration Samples

To prepare stock solutions of ciprofloxacin (CIP) and ornidazole (ORN) at concentrations of 10 mg/50 mL, each compound was accurately weighed and separately dissolved in methanol within a 50 mL volumetric flask. The final volume was adjusted to the mark with methanol, and sonication was applied for a few minutes to facilitate dissolution.

Calibration solutions were prepared by diluting the stock solutions to achieve concentrations ranging from 3.0 to 24.0 µg/mL for CIP and 6.0 to 32.0 µg/mL for ORN. Each calibration solution was prepared in a 10 mL volumetric flask. To create an acidic medium, 2 mL of 0.1 M HCl was added to each flask, and the final volume was adjusted to the mark with methanol. All UV spectra of these prepared samples in an acidic methanolic medium were recorded.

Preparation of Validation Samples

To validate the method, three sets of laboratory-prepared solutions were used. The first set consisted of 11 binary synthetic mixtures of the target drugs, prepared daily to ensure the accuracy and precision of the spectrophotometric method. The second set included intra-day and inter-day samples of ciprofloxacin (CIP) at concentrations of 6.0, 12.0, and 18.0 µg/mL, and ornidazole (ORN) at concentrations of 8.0, 16.0, and 24.0 µg/mL, across three different concentration levels. This set was designed to assess the accuracy and precision of the assay results obtained by the method. The third set consisted of standard addition samples, where drug standards were added to the solution of the commercial sample (ORCIPOL®) at a constant concentration. For CIP, the standard concentrations were 5, 10, and 15 µg/mL, and for ORN, the concentrations were 6, 12, and 18 µg/mL. This set was used to evaluate the selectivity of the method. To determine the recovery of the added standards in the standard addition samples, a solution of the commercial tablet sample, which did not contain drug standards, was prepared. Each sample solution was prepared in a 10 mL volumetric flask in the validation procedures. In preparing the solutions, 2 mL of 0.1 M HCl was added to each flask to create an acidic environment, and the final volume was adjusted to the mark with methanol.

Preparation of Tablet Sample

Ten ORCIPOL® tablets were finely powdered and homogenized. A quantity equivalent to one tablet (500 mg of each drug) was weighed and transferred to a 100 mL volumetric flask. After placing the flask in an ultrasound bath for 30 minutes, methanol was added to bring the solution to the mark. The resulting solutions were then carefully filtered through a 0.20 µm membrane filter (Sartorius Minisart, Hannover, Germany). The filtered solution was diluted to the CIP and ORN working ranges using the same solvent. In this dilution step, each sample solution was prepared in a 10 mL volumetric flask. In preparing the solutions, 2 mL of 0.1 M HCl was added to each flask to create an acidic environment and the final volume was adjusted to the mark with methanol. This sample preparation process was repeated ten times to ensure consistency. The UV spectra of the prepared samples were recorded.

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