

ICH-compliant Spectrophotometric Analysis of Zonisamide and Naltrexone Hydrochloride In Combined Formulations

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Abstract

A validated spectrophotometric technique for the quantification of Zonisamide and Naltrexone hydrochloride in both pure drug and synthetic combination formulations was described. This analytical approach employs the zero-crossing technique, offering a straightforward, sensitive, accurate, and reproducible means of analysis. In this procedure, zonisamide exhibited a significant derivative absorbance at 267.2 nm (zero-crossing point for naltrexone hydrochloride). Conversely, Naltrexone hydrochloride was quantified at 238.4 nm (zero-crossing point of zonisamide). The method demonstrated excellent linearity for both compounds within a concentration range of 3–21 µg/ml. The method was validated in accordance with the International Council for Harmonization (ICH) Q2(R2) guidelines, confirming its suitability for routine quality control in bulk drugs, mixtures, and formulations containing these two active ingredients.

Keywords: Derivative, Naltrexone hydrochloride, Simultaneous, Spectrophotometry, Validation, Zonisamide.

1. Introduction

Zonisamide (ZON) is a compound classified as a 1,2-benzoxazol-3-yl methane sulphonamides (Figure 1) and is categorized as an antiepileptic drug. As a relatively recent addition to antiepileptic treatment, it modifies the fast deactivation threshold of voltage-dependent Na-channels. This mechanism decreases recurring firing of action potentials at high frequencies, thereby aiding the prevention of seizures. Furthermore, ZON restrains low-threshold T-type Ca-channels in neurons, potentially impeding the propagation of seizures between the cells. ZON is commonly employed in the management of epilepsy and the prevention of seizures and is often prescribed as an adjunct therapy alongside other medications to address specific seizure types in individuals with epilepsy. It is classified as an anticonvulsant or antiepileptic drug category (Tripathi, 2013). ZON is approved by Central Drugs Standard Control Organisation (CDSCO) in 2005 as anti-epileptic drug. Naltrexone hydrochloride (NAL), depicted in Figure 2, is chemically identified as 4-(cyclopropylmethyl)-3-(4a,9-dihydroxy-2,4,5,6,7a,13-1H-4,12-methanobenzofuro [3,2-e] isoquinolin-7-one. It functions as an opioid

antagonist by blocking the mu-opioid receptor, thereby providing therapeutic benefits for the treatment of alcohol and opioid dependence. NAL is frequently used in medications for depression and epilepsy, either as a monotherapy or in combination with other drugs (Tripathi, 2013). NAL is approved by Central Drugs Standard Control Organisation (CDSCO) in 1996 in the treatment of narcotic antagonist and alcohol dependence. High-performance liquid chromatography (HPLC) has been recognized as an official method for analyzing ZON (The Indian, 2018, United, 2020). Spectrophotometry and HPLC have been employed to assess ZON independently (Yamini, 2029; Firake, 2020; Sonawane, 2022; Nimbokar, 2021; Meyyanthan, 2022; Lourenco, 2017). The official determination of NAL has been conducted using HPLC and titrimetric methods (The Indian, 2018; British, 2020; The European, 2025; United, 2020). Spectrophotometric and HPLC methods have been used for the individual analysis of NAL (Kossoski, 2013; Ahmed, 2020; Sontakke, 2023; Golnoz, 2019; Pekka, 2012; Sarasambi, 2010). Both ZON and NAL can be simultaneously analyzed with other molecules using spectrophotometric and HPLC techniques (Patel, 2017; Shah, 2019; Paleti,

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2016; Shah, 2017; Patel, 2015; Patel, 2019). This paper presents a sensitive and reproducible derivative spectroscopic method (Buckett, 1988) for estimating ZON and NAL in combination.

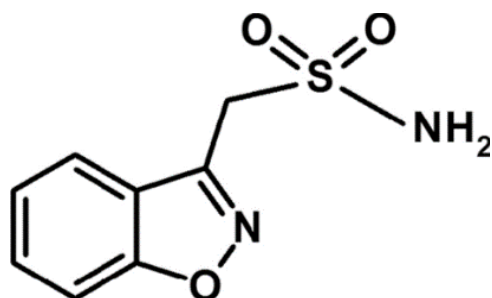


Figure 1: Structure of Zonisamide

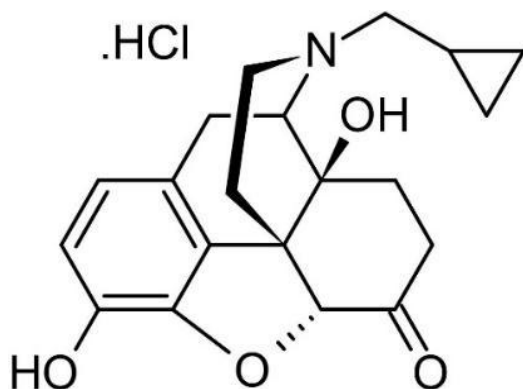


Figure 2: Structure of Naltrexone hydrochloride

MATERIAL AND METHODS

Instrumentation

In the analytical procedures, a Shimadzu 1800 double beam UV-Visible spectrophotometer, equipped with UV Probe 2.0 software, a Sartorius CP224S analytical balance, and a Frontline FS 4 ultrasonicator, were employed.

Chemicals and reagents

Naltrexone hydrochloride (NAL) and Zonisamide (ZON) were procured from Intas Pharmaceuticals Ltd. with purity of 99.34% & 99.76%, respectively. All chemicals and reagents which were used in this study were of AR grade and obtained from Merck Chemicals, India.

Preparation of solutions

Preparation of stock and standard solutions

Individual standard stock solutions, each containing 10 mg of ZON or NAL, were independently prepared by dissolving the respective reference standards in methanol in separate volumetric flasks. Subsequently, a

working standard solution was formulated by diluting the ZON and NAL standard solutions to an appropriate volume. A calibration curve for solutions containing 3-21 µg/ml of both ZON and NAL was independently established.

Preparation of laboratory mixture

A laboratory mixture with a total mass of 175 mg was formulated by combining 32 mg of NAL and 91 mg of ZON with 59 mg of commonly used capsule excipients, including talc, magnesium stearate, starch, and lactose.

Preparation of test solution

A precisely quantified amount of the synthetic blend, comprising 30 mg of ZON and 10.55 mg of NAL, was dissolved in methanol. The solution was subjected to ultrasonication for 10 min to ensure complete dissolution, followed by filtration through Whatman filter paper. From this filtered solution, 10 ml was further diluted to 100 ml with methanol, yielding a test solution with concentrations of 30 µg/ml ZON and 10.55 µg/ml NAL. A working solution was subsequently prepared by diluting an appropriate portion of the test solution to achieve final concentrations of 9 µg/ml ZON and 3.16 µg/ml NAL.

Derivative spectrophotometric method

The ultraviolet absorption spectra offer ZON and NAL, each at a concentration of 10 µg/ml in the selected diluent, were scanned within the 200–400 nm range. The zero order UV spectra obtained were mathematically converted into first-order derivative spectra (D_1) using a delta lambda ($\Delta\lambda$) of 1.0 and a scaling factor of 4.0. The zero-crossing wavelengths were identified as 238.4 nm for ZON and 267.2 nm for NAL. Consequently, ZON was measured at 267.2 nm, the zero-crossing point for NAL, whereas NAL was assessed at 238.4 nm, the zero-crossing point for ZON.

Validation of method

The developed method was validated in accordance with the ICH Q2R2 guidelines (ICH Q2(R2), 2023) by assessing key parameters, including limit of detection (LOD), limit of quantification (LOQ), accuracy, linearity, and precision.

Linearity

A series of calibration standards containing ZON and NAL was prepared, at concentrations ranging from 3 to 21 µg/ml. The first-order derivative (D_1) response for all working standards was measured at 267.2 nm for ZON and 238.4 nm for NAL, utilizing the appropriate solvent as a blank. Calibration graphs were constructed by plotting D_1 absorbance values against their respective concentrations, and the correlation coefficients (r^2) were calculated to assess the method's linearity.

Accuracy

With the help of recovery studies and standard addition method accuracy of the method was confirmed. A

previously analysed sample was supplemented with known quantities of ZON and NAL at concentrations of 80%, 100%, and 120%.

Precision

The method precision was checked by repeatedly measuring and recording the first-order derivative absorbance of the ZON (15 µg/ml) and NAL (5 µg/ml) solutions six times ($n = 6$) without altering any instrument settings. To assess intraday and interday precision, standard solutions of ZON and NAL at concentrations of 12, 15, and 18 µg/ml were analysed. The intraday precision involved analysing each concentration three times within a single day, whereas the interday precision was determined by conducting the same analysis over three consecutive days. The results are reported as the relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantitation (LOQ)

According to the ICH Q2R2 guidelines, signal-to-noise (S/N) ratios of 3.3:1 and 10:1 were calculated.

Analysis of ZON and NAL in powder mixture

The amounts of ZON and NAL in the mixture were calculated using the calibration curves regression equations.

RESULTS AND DISCUSSION

Method

In the proposed method, two analytical wavelengths (267.2 nm for ZON and 238.4 nm for NAL)

were used to estimate both drugs from the mixture. (Figure 3).

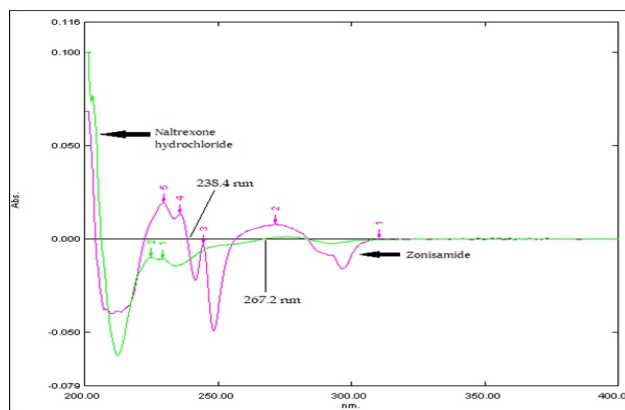


Figure 3. First Order Derivative Spectra of ZON and NAL (10 µg/ml)

Method validation

Linearity

The method was assessed to verify its suitability for the intended application and its compliance with the validation standards. A calibration curve was used to determine the correlation coefficient, indicating a linear response within a range of 3-21 µg/ml for both ZON and NAL. Table 1 provides a summary of the data obtained from the linearity study.

Table 1: Linearity results of the method

S. N.	PARAMETERS	ZON	NAL
1.	Linearity range	3 - 21 µg/ml	3 - 21 µg/ml
2.	Regression equation	$y = 0.0007x + 0.00007$	$y = 0.001x + 0.0004$
3.	Slop	0.0007	0.001
4.	Intercept	0.00007	0.0004
5.	Correlation coefficient (r2)	0.9994	0.9993

Accuracy (%recovery)

The recovery values obtained for ZON (99.87-100.6%) and NAL (99.54-100.9%) reflects the accuracy of the method.

Precision

Table 2 shows the %RSD values pertaining to the repeatability data for ZON and NAL. The low %RSD values, all below 1%, demonstrated the repeatability of the proposed method. For intra-day precision, the %RSD ranged from 0.56-0.84 ZON and 0.67-0.91 NAL. In

contrast, the interday %RSD values ranged from 0.94 to 1.12 for ZON and from 1.08 to 1.24 for NAL, underscoring the precision of the method.

LOD and LOQ

The method reflected very good sensitivity, with the LOD established at 0.76 µg/ml for ZON and 0.41 µg/ml for NAL. The LOQ were determined to be 2.30 µg/ml ZON and 1.25 µg/ml NAL. Table 2 summarizes the validation parameters for the derivative spectrophotometric method.

Table 2. Summary of validation parameters

S. N.	PARAMETERS	RESULTS	
		ZON	NAL
1.	Wavelengths (nm)	267.2	238.4
2.	Beer's law limit (µg/ml)	3-21	3-21
3.	Accuracy (% Recovery, n = 3)	99.87-100.6	99.54-100.9
4.	Precision		
	Repeatability (% RSD, n = 6)	0.62	0.79
	Intraday (% RSD, n = 3)	0.56-0.84	0.67-0.91
	Interday (% RSD, n = 3)	0.94-1.12	1.08-1.24
5.	LOD (µg/ml)	0.76	0.41
6.	LOQ (µg/ml)	2.30	1.25

%RSD = percentage relative standard deviation, n = number of determinations

Assay of the mixture

The method demonstrated efficacy in analysing ZON and NAL within a powder mixture, with the assay results corroborating the labelled claim (Table 3). This newly developed and validated method is considered

appropriate for estimating ZON and NAL in a combined synthetic formulation and can be employed for assaying their formulation. This method, characterized by its straightforwardness, cost-efficiency, accuracy, precision, rapidity, and sensitivity, accurately determines the ZON and NAL in a powder mixture, rendering it ideal for routine quality control evaluations.

Table 3. Assay of synthetic mixture

Dosage form	Label Claim	% Assay ± S.D. (n=6)
Powder mixture	ZON – 91 mg	99.93 ± 1.24
	NAL – 32 mg	100.1 ± 1.06

S.D. = standard deviation, n = number of determinations

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