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SHORT COMMUINCATION article

Study of the diastereoisotopic protons effects on the stability of dorzolamide stereochemical by spectroscopic methods

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Abstract: In scientific and analytical research, Ultraviolet-visible spectroscopy, Fourier Transform Infrared spectroscopy, and Nuclear Magnetic Resonance spectroscopy have widely been used to analyze and characterize various substances. Ultraviolet, infrared, and Nuclear Magnetic Resonance spectroscopy were studied for diastereoisotopic protons of dorzolamide, which is used to treat glaucoma and ocular hypertension. Methanol was used as a solvent in Ultraviolet and we obtained three wavelengths: 203 nm, 253 nm, and 257 nm, where Ultraviolet spectroscopy was employed for stability testing of pharmaceuticals. By analyzing the Fourier Transform Infrared spectrum, we also can determine the presence or absence of specific functional groups in a compound, helping to identify the compound or confirm its structure in analyzing the Nuclear Magnetic Resonance spectrum. It can determine the types and numbers of hydrogen (proton) and carbon atoms in a molecule and their chemical environment, which helps elucidate the molecular structure.

Introduction

Chemically, dorzolamide (DZL) is (4S,6S)-4 (ethylamino)-6-methyl-5,6-dihydro-4H-thieno[2,3-b] thiopyran- 2-sulfonamide 7,7-dioxide hydrochloride (**Figure 1**) [1, 2]. DZL is a carbonic anhydrase inhibitor, which is used for the treatment of glaucoma and ocular hypertension [2, 3]. After topical ocular administration, DZL rapidly reaches the systemic circulation where it distributes preferentially to red blood cells [4]. Glaucoma is a multifactorial optic neuropathy marked by progressive degeneration from the loss of optic nerves and retina ganglion cells. Another strategy to reduce intraocular pressure is to increase the drainage of ocular fluid through the trabecular meshwork pathway (cholinergic agonists and prostaglandin analogs) [4]. Glaucoma is the leading cause of irreversible global blindness; the two drugs are often combined in eye drops for the therapy of glaucoma [5-7]. Clinical studies were conducted using the single stereoisomer (I) with the absolute configuration SS around the two chiral centers of the molecule [8-10]. In order to assess the potential for the *in-vivo* inversion of configuration at one or both chiral centers of (I) and the stereochemistry and an absolute configuration of the

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metabolite [4, 11, 12]. A literature scan showed that various studies were reported for the analysis of DZL [1], including spectroscopy of Ultraviolet (UV), Infrared (IR), and Nuclear Magnetic Resonance (NMR). UV-visible spectroscopy is a technique used to study the interaction of light with a sample in the UV and visible regions of the electromagnetic spectrum [13, 14]. It provides information about the electronic transitions of molecules and is commonly used to determine the absorption or transmission of light by a sample at different wavelengths [2, 15]. IR spectroscopy is an analytical technique used to study the molecules in the IR light absorption function. It can be used to analyze the chemical structure and DZL properties, as well as to detect impurities [16]. NMR of DZL is a technique used to analyze the molecule structure of this drug. The NMR allows us to determine the positions and interactions of atoms in the DZL molecule the chemical liaisons and the molecular conformations [17]. This study aims to determine the stereochemical of DZL utilizing UV, FTIR, and NMR spectroscopy. This investigation was used to identify the drug's diastereoisotopic proton.

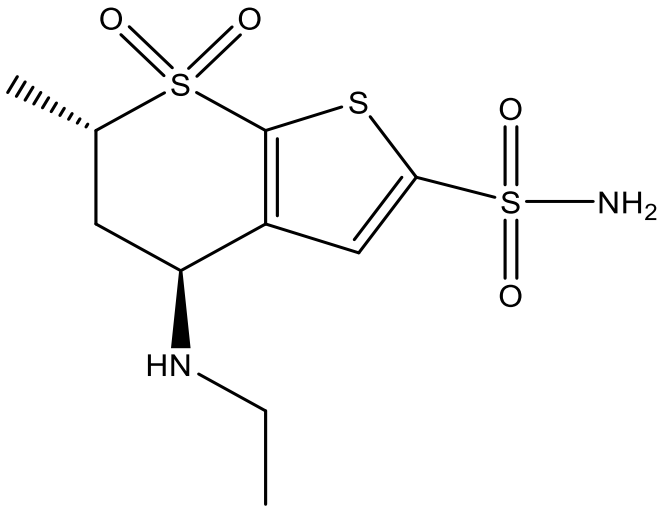

Figure 1: Chemical structure of dorzolamide

Materials and methods

Dorzolamide can be characterized using UV, IR, and NMR spectroscopy methods. The following are the general steps for each technique: The apparatus utilized in this investigation was of the type “SPECORD 50 PLUS” brand, this new dual-beam UV/Vis photometer for the spectral range from 190 to 1100 nm combined. The commercially available eye drops containing a 2.0% solution of sterile DZL HCl (20 mg/ml) were used. Prepared as a solution of DZL in a methanol solvent, a UV spectrophotometer is used to measure the absorbance of the solution in the 200-400 nm wavelength range. Analyze the UV absorption spectrum to identify characteristic peaks and determine the concentration of DZL. The Fourier Transform Infrared spectroscopy (FTIR) "Cary 630 FTIR" equipment was used in this study. Analyze the Chantillon using an IR spectrometer to register the IR absorption spectrometer, then analyze the specter to identify the bands with DZL absorption characteristics reveal information about the molecule structure, and better understand the chemical structure of organic compounds and make informed decisions about their properties, reactivity and behavior in various environments. We used the software, master nova, this software allows us to automate the process of analyzing and predicting NMR spectra of organic molecules by entering their structural information.

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Results and discussion

UV-vis spectroscopy analysis: The specter of absorption UV-Vis of DZL in methanol presents three characteristic bonds of absorption as shown in **Figure 2** and **Table 1.**

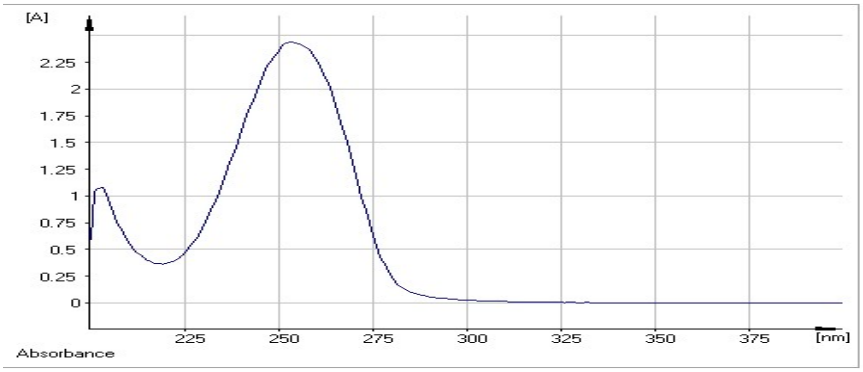

Figure 2: UV- absorbance spectra of dorzolamide

According to the Beer-Lamber absorption law, we can determine the molecular extinction coefficients which give information on the intensities of the absorption bands. The first bond situated at λ_{max}=203 nm with an energy of Δ_E=140.967 kcal/mol attributed to electronic transition $\pi \rightarrow \pi^*$ which corresponds to the double links between both C=C. The second bond situated at λ_{max}=253 nm an energy of about Δ_E=113.108 kcal/mol attributed to electronic transition $\pi \rightarrow \pi^*$ which corresponds to the double links between both S=O. The third bond situated at λ_{max}=257 nm with an energy about Δ_E=111.348 Kcal/mol attributed to electronic transition $n \rightarrow \pi^*$ which corresponds to the double bond and free doublet of heteroromes S=O, C-N.

Table 1: UV-Vis spectroscopic analysis of dorzolamide

	Bond I	**Bond II**	**Band III**
λ_{max}	203	253	257
A	1.079	2.438	2.395
ΔE	140.967 kcal/mol	113.108 kcal/mol	111.348 Kcal/mol

In **Figure 3**, it can be noticed that the electronic transition energy is inversely related to the wavelength, so when the transition energy decreases, the wavelength increases. This observation provides information on the molecule's stability based on the number of existing intramolecular interactions (electronic and structural).

The infrared spectrum presents 8 characteristic absorption bands: Two thin bands are located respectively at 2890 and 2950 cm^{-1} corresponding to the symmetric and asymmetric valence vibrations of the bonds of CH methyl (**Table 2**). The presence of sulfur in the structure of this product is characterized by a band located around 1166 cm^{-1} corresponding to the valence vibration of the C-S bond. A wide bung around 3304 cm^{-1} is attributed to the valence vibration of the N-H intramolecular hydrogen bond. The amine function is characterized by a fine absorption bung around 1636 cm^{-1} corresponding to the deformation vibration. A fine absorption bung is located at 1630 cm^{-1} attributed to the valence vibration of aromatic C=C. The aromaticity of this product is confirmed by the presence of an absorption band around 3010 cm^{-1} corresponding to the valence vibration of aromatic C-H, and an absorption gap is located at 620 cm^{-1} corresponding to the deformation vibration of C-H outside the plane (**Figure 4**).

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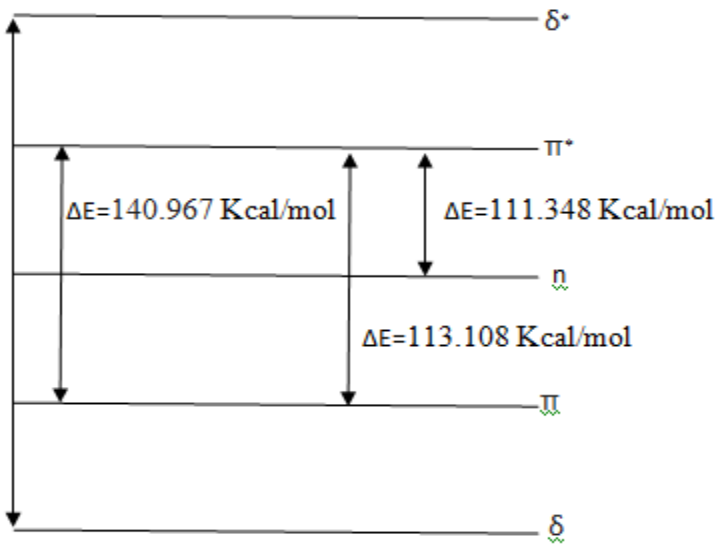

Figure 3: Energy diagram of dorzolamide

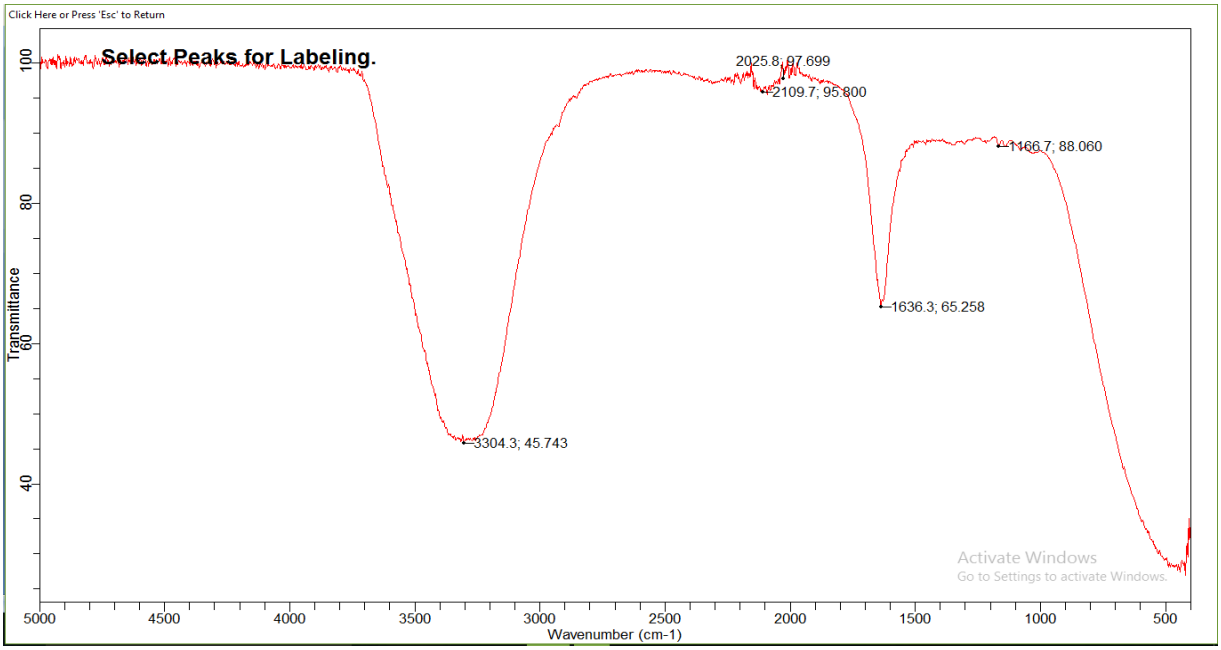

Figure 4: Dorzolamide IR spectrum

Table 2: Result of IR spectroscopic analysis of dorzolamide

	ν (cm^{-1})	δ (cm^{-1})	γ(cm^{-1})
CH_3	2890,2950	-	-
C=C aromatic	-	1630	-
C-H aromatic	-	-	620
C-S	1166	-	-
N-H amine	3304	1636	685

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H^1 NMR spectroscopic analysis: The determinations of the structure of DZL, based on the H NMR spectra indicate the presence of 09 carbon atoms.

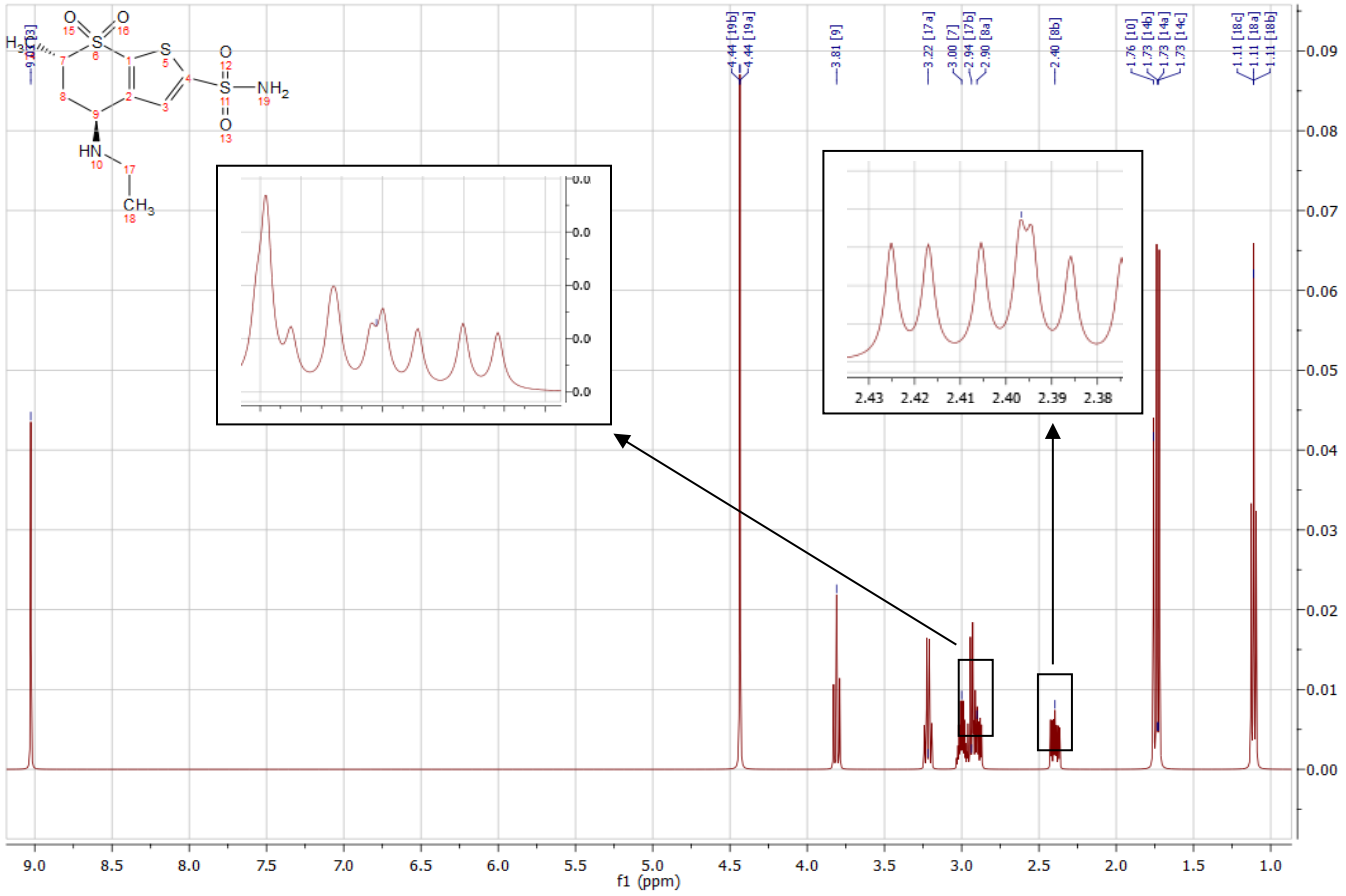

Figure 5: H MNR spectrum of dorzolamide

Table 3: Chemical shifts in H NMR and the attribution of dorzolamide signals

Position	RMN^{1}H
3	9.03(**s, 1H**)
7	3.00 (**s, 1H**)
8a	2.90(**d, 2H**)
8b	2.90(**d, 2H**)
9	3.81 (**s, 1H**)
14	1.73(**m, 4H**)
17a	3.32(**s, 1H**)
17b	3.32(**s, 1H**)
18	1.11(**m, 3H**)
NH	1,76(**m, 1H**)
NH_2	4.44(**m, 2H**)

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H^1NMR spectrum analysis of DZL shows the presence of multiple protons corresponding to a group (NH) at 1.76 ppm, and 4.44 ppm. The signals at 1.11, 1.73, 3.32, 3.81, 9.03 ppm are attributable respectively to (H-18, H-14, H-17, H-9, H-3). A singular signal of a proton resonating at 3.00 ppm is attributable to the (H-7) presence of methyl. Two multiply signals resonating at 2.90 ppm attributable to (H_{8a}, H_{8b}) (ddd,$^2J_{ab}$, $^3J_{ac}$, $^3J_{ad}$, 2H), (ddd,$^2J_{ba}$, $^3J_{bc}$, $^3J_{bd}$, 2H) presence of diastereoisotopic protons.

Conclusion: UV spectroscopy was used to assay the absorption of light by DZL in the ultraviolet range which provides valuable information about the chemical structure and electronic transitions in the pharmaceutical form. The absorption maxima were 203, 253, and 257 nm. IR spectroscopy was used to analyze the infrared radiation absorbed by DZL which allowed the identification of the functional groups, providing information about the molecular structure, such as the presence of carbonyl groups, amines, and aromatic rings. NMR spectroscopy, specifically proton HNMR, was used to analyze the NMR of protons in the DZL molecule which provides information about the connectivity of the atoms in DZL, allowing for the determination of the chemical environment and molecular structure. Thus, by employing these spectroscopic techniques, were able to spectroscopy to assay the diastereoisotopic protons of DZL.

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Author contribution: NB conceived and designed the study. HOA collected data. HOA & KS analyzed data. HOA & NB performed data analysis and interpretation. HOA & NB drafted and revised the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for its contents.
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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission were completely observed by the authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.