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A complete study of Doxazosin characterization

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Doxazosin is an important drug used to treat hypertension and prostatic hyperplasia. An enantiomeric (*R*)/(*S*) doxazosin mesylate mixture was complete characterized by NMR and additionally FT-IR spectroscopy techniques. Different NMR experiments were performed, such as APT, HSQC, and HMBC in order to confirm the NMR signals assignments. All the hydrogens and most of carbons atoms were assigned. In this context, the results reported here, consists in important information for identification and quality control of doxazosin mesylate.

Keywords: doxazosin; NMR; FT-IR; signals assignment.

Introduction

Doxazosin ((4-(4-amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl)(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methanone) is a long-acting selective α_1-adrenergic antagonist, employed in treatment for arterial hypertension (1) and benign prostatic hyperplasia (2). Pfizer introduced tablets of doxazosin mesylate in US market in 1995 (3). The action of doxazosin in hypertension is associated with their effect in reducing total peripheral resistance by selective postsynaptic α_1-blockade, without affecting cardiac output, and heart rate (1). Furthermore recent findings suggested that doxazosin may become a new pharmacotherapy alternative for the treatment of gliomas (4).

Advantages of repurposing drugs are the well-defined pharmacokinetics and side effects, and the drug has passed the required toxicity and safety tests with settled protocols and dosing (5). Regarding doxazosin, it is established the drug's antitumoral effects are not related with its α1-adrenoceptor antagonism (6). Due to its physicochemical characteristics, doxazosin is able to permeate the blood–brain barrier (7), and we found the drug presented low neurotoxicity on non-tumor cells (4).

The chemical structure of doxazosin is formed by a quinazoline core A, and a 1,4-benzodioxane core B, linked by a piperazine ring (**Figure 1**). The quinazoline core linked to piperazine is a very important scaffolding in doxazosin structure, considering that it is ubiquitous in their prazosin and terazosin analogues (8). Despite the importance of this drug in therapeutic use, there are few reports in the literature about their chemical characterization (9), which makes it difficult to control their quality. The existence of spectroscopy data about doxazosin characterization, may be a useful guide for impurities and degradation products identification in doxazosin mesylate. Considering this aspect, we report here the first detailed analysis describing the complete NMR, using bi-dimensional techniques for the characterization of doxazosin mesylate in the racemate form, in which the Infrared spectrum FT-IR was used.

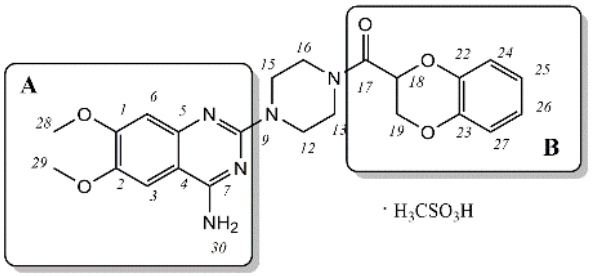

Figure 1 Structure of rac-doxazosin mesylate.

Methodology

An enantiomeric (*R*)/(*S*) doxazosin mesylate mixture, purchased from Nifty Labs PVT LTD, was solubilized in DMSO-d_6 (20 mg in 0.5 mL) and the nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) was recorded in a Bruker Ascend spectrometer with standard pulse sequences operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts were reported as relative values (ppm) to TMS.

The NMR multiplicities *s*, *d*, *t*, *q*, and *m* stand for singlet, doublet, triplet, quartet and multiplet, respectively. The ^{1}H NMR AB systems were presented in the following order: hydrogen a (Ha) the most deshielded and hydrogen b (Hb) the most shielded. For validation of these assignments, theoretical calculations of ^{13}C NMR chemical shifts were performed in a software MestReNova 6.0.2-5475 (MestreLab Research S.L., 2009). FT-IR spectra was recorded in a Perkin Elmer Spectrometer BXII using an ATR probe.

Results and Discussion

NMR experiments

The quinazoline ring A (see **Figure 1**) presents two singlets corresponding the aromatic hydrogens H_3 and H_6 in 7.24 ppm and 7.65 ppm, respectively. In relation to the ring substituents, the two singlets corresponding to methoxy groups C_{28} and C_{29} appear in 3.84 and 3.90 ppm and can be interconverted. The two hydrogens linked to nitrogen at C_7 produce two individual broad singlets in 8.70 and 8.81 ppm. The ^{1}H NMR chemical shifts and signals multiplicities were shown in **Figure 2** were and summarized in **Table 1**.

The signals corresponding to aromatics C_3 and C_6 of quinazoline ring appear in, respectively, 99.03 and 104.76 ppm (**Figure 3**), and these carbons can be correlated between H_3 and H_6 in HSQC experiment. Between C_3 and C_6 signals, there is the C_4 signal, and its assignment is confirmed by HMBC spectra, considering its ^{2}J coupling with H_3. The C_1 and C_2 atoms generated the signals at 155.44 ppm and 146.92 ppm respectively, an characteristic of aromatic quinazoline carbons di-methoxy substituted found in literature (10). The C_9 chemical shift (151.39 ppm) was attributed considering that this signal in HMBC experiment has no correlation with hydrogens of quinazoline ring. Additionally, the two methoxyl groups, C_{28} and C_{29}, substitutions on ring A, produces only one signal at 56.18 ppm. Considering the HMBC analysis the carbons C_4, C_5 and C_7 were assigned as chemical shifts in 101.66, 135.99 and 161.32 ppm respectively. The ^{13}C NMR chemical shifts and correlations found in HSQC and HMBC spectra can be observed in **Table 2**, while the NMR bi-dimensional spectra are showed in **Figures 4** and **5**.

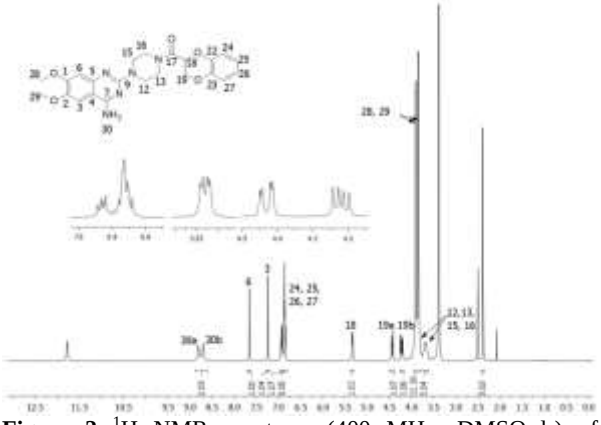

Figure 2 ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of doxasozin.

Table 1 ^{1}H NMR data for doxazosin.

δ (ppm)	Multiplicity	Assignment
3.54	Multiplet	H_{13}, H_{16}
3.99	Multiplet	H_{12}, H_{15}
3.84	Singlet	H_{28} or H_{29}
3.90	Singlet	H_{28} or H_{29}
4.22	double duplet *J* = 11.8 Hz; 6.5 Hz	H_{19b}
4.43	double duplet *J* = 11.8 Hz; 2.5 Hz	H_{19a}
5.33	double duplet *J* = 6.5 Hz; 2.5 Hz	H_{18}
6.83-6.95	multiplet	H_{24}, H_{25}, H_{26} H_{27}
7.24	singlet	H_3
7.65	singlet	H_6
8.70	broad singlet	N-H_b
8.81	broad singlet	N-H_a

The 1,4-benzodioxane ring B signals have four aromatic hydrogens H_{24}, H_{25}, H_{26}, H_{27} that appear in upfield compared to quinazoline. The aromatic hydrogens signal, characteristic of a 1,4-benzodioxane ring (11, 12), was non-resolved in the range of 6.83-6.95 ppm. The three aliphatic hydrogens generated the signals at between 4 to 6 ppm. The diastereotopic hydrogens H_{19a} and H_{19b} appeared at 4.43 ppm and 4.22 ppm respectively, while the hydrogen H_{18} linked to chiral carbon C_{18} produced a double of doublets at 5.33 ppm with $J_{H18H19b}$=6.5 Hz and $J_{H18H19b}$=2.5 Hz. The multiplicity of H19b presented a double of doublets with J_{H19H18}=2.5Hz and a vicinal coupling $J_{H19aH19b}$=11.8 Hz. Due to their spatial disposition, these hydrogens coupling in a different manner with H_{18}, produceding J values of 6.8 and 2.5 Hz. This coupling between these hydrogens justifies completely the multiplicity of signals presents in 4.22, 4.43 and 5.33 ppm.

In relation to the ^{13}C NMR spectra, the 1,4-benzodioxane ring carbons C_{18} (CH) and C_{19} (CH_2) produce the signals at 69.44 and 64.66 ppm respectively, and its assignment can be confirmed in APT and HSQC spectra (**Figure 3** and **Figure 4**). A ^{2}J coupling of C_{19} with H_{18}, and C_{18} with H_{19a} and H_{19b} diasterotopic hydrogens can be also observed in HMBC spectra. The aromatic carbons of 1,4-benzodioxane (C_{24}, C_{25}, C_{26} and C_{27}) produced four undifferentiated signals between 115 to 120 ppm. C_{22} and C_{23} carbons produced two signals at 142.84 and 143.06 ppm, assignment that can be confirmed by coupling with aromatic hydrogens of 1,4-benzodioxane in HMBC spectra. In 165.27 ppm, it is present the only carbonylic carbon of the structure, which has a ^{2}J with H_{18} and ^{3}J coupling with and H_{19} in HMBC spectra. The analysis of HSQC spectra showed that hydrogens

H_{19a} and H_{19b} are linked to the carbon at 64.66 ppm and the H_{18} with the carbon at 69.44 ppm. In addition, it may be verified that methoxy groups C_{28} and C_{29} produced only one signal at 56.18 ppm.

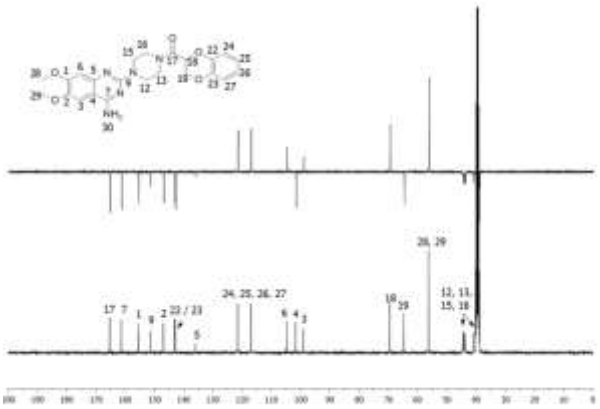

Figure 3 Full and APT ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of doxazosin mesylate.

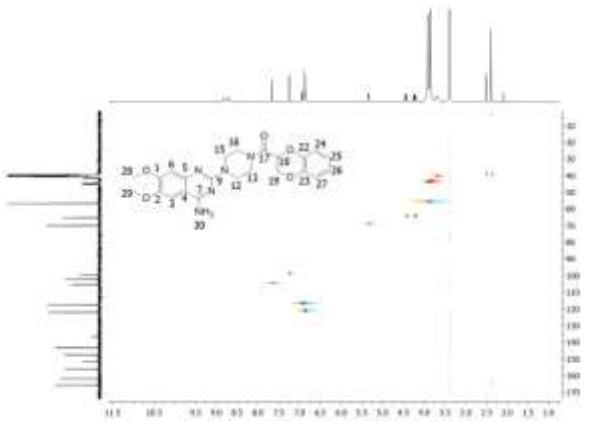

Figure 4 HSQC NMR spectrum of doxasozin mesylate (DMSO-d_6).

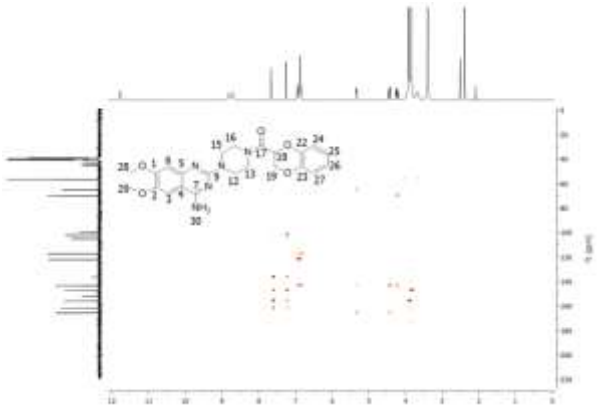

Figure 5 HMBC NMR spectrum of doxasozin mesylate (DMSO-d_6).

Table 2 ^{13}C NMR data for doxazosin mesylate and correlation of ^{1}JCH observed in HSQC and ^{n}JCH in HMBC

C	δ ^{13}C (APT)	$^{1}J_{CH}$ HSQC	$^{n}J_{CH}$ HMBC
C_1	155.44	-	H_3, H_6, $H_{28/29}$
C_2	146.92	-	H_3, H_6, $H_{28/29}$
C_3	99.03	H_3	-
C_4	101.66	-	H_3
C_5	135.99	-	H_3, H_6
C_6	104.76	H_6	-
C_7	161.32	-	H_3, H_6
C_9	151.39	-	-
C_{12}, C_{13}, C_{15}, C_{16}	40.80; 43.80; 44.05; 44.46	H_{12}, H_{13}, H_{15}, H_{16}	-
C_{17}	165.28	-	H_{18}, H_{19a}
C_{18}	69.44	H_{18}	H_{19b}
C_{19}	64.66	H_{19a}, H_{19b}	H_{18}
C_{22}, C_{23}	142.84; 143.06	-	H_{24}, H_{25}, H_{26}, H_{27}, H_{18}, H_{19a}, H_{19b},
C_{24}, C_{25}, C_{26}, C_{27}	116.92; 117.03; 121.45; 121.56	H_{24}, H_{25}, H_{26}, H_{27}	H_{24}, H_{25}, H_{26}, H_{27}
C_{28}, C_{29}	56.18	-	-

The piperazine ring presented signals characteristics of the presence of piperazine rings (13, 14) with two multiplets corresponding the two groups of equivalents methylene hydrogens H_{12} and H_{15} centered at 3.99 ppm, and H_{16} and H_{13} at 3.54 ppm, which is superposed to singlets from hydrogens of methoxyl 28 and 29. In relation to the NMR ^{13}C, the four carbons of piperazine linker appeared at 40 to 45 ppm, and due to the similarity of the chemical environment, the assignment of these carbons is very challeging.

Considering that the mesylate salt of doxazosin was analyzed, it is possible to observe that when the methyl group of mesylate is at 2.38 ppm and in 11.60 ppm, the acid hydrogen is presented in H_3CSO_3H.

It was found a higher r^2 value (0.9915) for correlation between calculated ^{13}C NMR chemical shifts against the experimental values. These results show that carbon chemical shift assignment is adequate for the doxazosin structure, and that prediction can be used as tool for analyze fitting of the assignments. The correlation graph is shown in **Figure 6**.

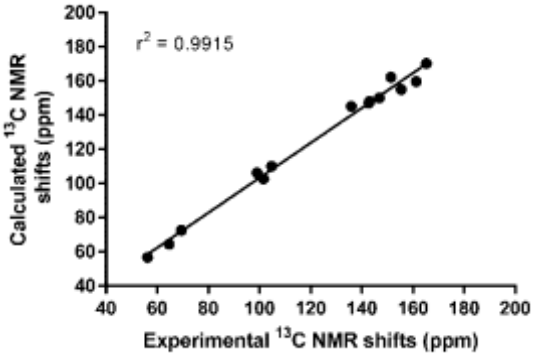

Figure 6 Correlation between calculated and observed NMR shifts. The carbons of piperazine ring and aromatic ring of 1,4-benzodioxane were not included in this analysis. The prediction of chemical shifts was performed using MestReNOva 6.0.2-5475 (MestreLab Research S.L., 2009).

The total assignments of NMR doxazosin may be used in quality control for characterization of impurities from doxazosin synthesis. In doxazosin monography present in British Pharmacopeia are listed 8 possible impurities that may be present in doxazosin (15). In addition United States Pharmacopeia recommends that the doxazosin mesylate production method must be evaluated to determine the potential formation of alkyl mesylates (16). Furthermore, the analysis of NMR spectra also contributes with important informations in relation to the presence degradation impurities.

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Infrared characterization

Some stretches are characteristic in doxazosin mesylate FT-IR spectra (**Figure 7**). The most important band in spectrum of doxazosin occurs in 1632 cm^{-1} attributed to the presence of tertiary amide band (C=O). Spectral broad band corresponding to N-H stretching of amine salt is localized at 3159 cm^{-1}. The strong band at 1595.48 can be attributed to N-H bending of aromatic amines. The four C-O bands (stretching) that are presented in doxazosin structure may appear in the region of 1259 to 1168 cm^{-1}.

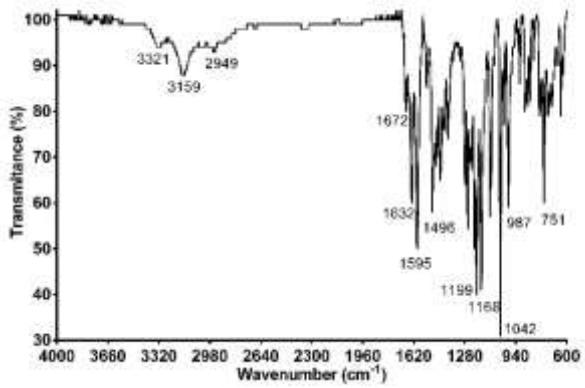

Figure 7 IR spectra (ATR) of doxazosin mesylate.

Conclusions

The mono- and bi-dimensional ^{1}H and ^{13}C NMR experiments performed here for doxazosin produced a considerable amount of new data. These data may be used as a tool to verify and prove structural modifications in doxazosin for future works. Additionally, the infrared spectra reported here is an important data for an easy doxazosin mesylate identification.

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