

*Full Length Research Paper*

# Ozonation process for hazardous drugs molecules in aqueous media

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**This study presents results of ozonation of some different aromatic rings of medicines in aqueous solution. The aim of the research was to investigate the influence of ozone on ring cleavage of drug molecules with an aromatic structure. In this regard, phenobarbital, naphazoline, doxycycline, isoniazid and salbutamol were chosen for exposure to ozone gas overnight. It is proved that ozone can attack the OH groups, opening the aromatic ring to form unsaturated acids, aldehydes and linear alkyl/cycloalkyl groups which then appear as stable products. It is suggested that the first step in these reactions includes penetration of ozone at the C-H bond with the formation of a quinone and a subsequent attack of the aromatic ring T-bond system by the ozone with the formation of ozonides. With this process, ozonation can produce simple, biodegradable and less carcinogenic compounds from complex aromatic ones.**

**Key words:** Ozone, aromatic compound, oxidation, toxicity.

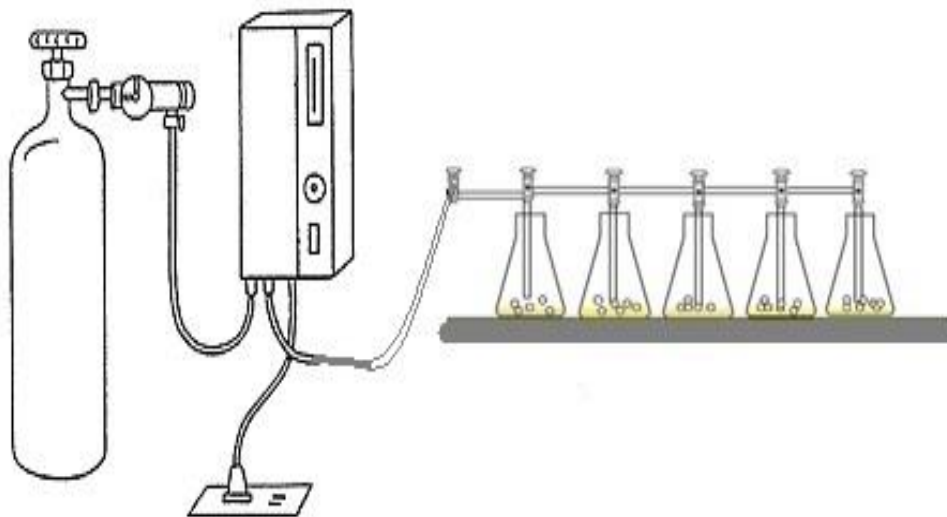
## INTRODUCTION

In recent years one of the most significant concerns of the research-based pharmaceutical industry is environmental contamination and, more specifically for pharmaceutical agents, safety. Among the organic compounds, soluble aromatics have been especially studied because of their biological stability (Dore et al., 1980). Aromatic rings are found in most drugs and have been studied widely due to their toxicity. Their potent toxicity or carcinogenesis is a major issue for human populations, as are their potential impact as environmental pollutants and in emissions from diesel

and gasoline engines. Relative to their importance, the toxicity, carcinogenicity and their metabolic pathways in organisms have been examined (Marie, 2009). They also preferred to be replaced with linear and branched alkyl/cycloalkyl groups.

Ozonation has been known for more than a century. In the 1920s, this phenomenon was studied while ozone-induced cracking was developed during and immediately after World War II; the research led to the discovery of the first chemical antiozonants. Ozone, a triatomic allotrope of oxygen, is a tremendously strong oxidant

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**Figure 1.** Schematic representation of batch reactor.

agent that can easily react with the unsaturated chemical bonds in organics. It also can cleave and oxidize benzene and other aromatic rings (Gong et al., 2008) (Sahu, 2011). The term “ozonolysis” as applied in this study specifically refers to the cleavage of bonds between  $sp^2$  or  $sp$  carbon atoms. The reaction between ozone and the C=C bond is assumed to follow a bimolecular law, where each C=C bond functions as an independent kinetic unit. The concepts behind this mechanism had been summarized previously (Anachkov et al., 2000; Anachkov et al., 1996; Razumovsky et al., 1986). Moreover, it also has been observed that ozonation can convert large molecules into smaller ones and increase the ratio of hydrophilic organics in water (Gong et al., 2008). Ozone is widely used in Europe for the removal of colors, tastes, odors and pathogens in drinking water.

Two mechanisms were considered for the reaction between the ozone molecule and an organic: (1) Direct  $O_3$  molecule oxidization (electrophilic addition) or (2) Indirect oxidization through  $\bullet OH$  (nucleophilic addition). Comparatively, the electron-donating groups (-OH, -NH etc.) react faster with  $O_3$  than the electron-withdrawing groups (-COOH,  $-NO_2$  etc.) when ozone attacks the ortho and para positions primarily. In the other words,  $O_3$  reacts easily with electron-donor with aromatic compounds resulting in ortho and para intermediates which can be further oxidized, consequently to quinonoid compounds and later to aliphatic compounds concluding with hydroxide radical and carboxy compounds. The following findings demonstrate the manner in which radicals like  $\bullet OH$  may be produced and the manner in which they function (Hui-xiang et al., 2005; Staehelin and Hoigne, 1982).

The literature concerning the kinetics and mechanism

of ozonation of such macromolecular compounds is scarce. Most qualitative opinions on the kinetics have been published (Marie, 2009; Sweeney, 1981). The problem is that ozonation is multistage process, and a wide variety of intermediates are formed that are difficult to identify.

## MATERIALS AND METHODS

### Sample preparation

All of the selected drugs were used as model to investigate the ability of the ozonation process to eliminate the aromatic rings of these compounds, with an ultimate goal of reducing their toxicity. Experiments were carried out in a glass reactor with jacket at room temperature, as illustrated in Figure 1. Ozone was produced for the test using an ozone generator, model CD- 0013.5. This unit produces 13.5 g of ozone per hour embedded in a flow of medical oxygen at  $3 \text{ L min}^{-1}$ . Depending on a solubility of the compounds, 50 mg of phenobarbital, naphazoline, doxycycline, isoniazid and salbutamol in deionized water in a volume of 50 ml were tested. The top of each glass reactor had five connections providing for the collection of materials with the same flow rate of the ozone bubbling. After ozonation, samples were collected, lyophilized and kept under refrigeration until characterization.

### Laboratory determinations

Fourier transform infrared spectroscopy (FTIR) analysis using a Perkin-Elmer spectrum GX spectrometer was used to determine the FTIR spectra of samples before and after ozonation. Lyophilized powders of the sample were mixed with spectrophotometric grade KBr at 1:5 ratios and pressed into pellet form. FTIR spectra were recorded in the frequency range of  $400$  to  $4000 \text{ cm}^{-1}$  with 32 scans and a resolution of  $4 \text{ cm}^{-1}$ . The functional groups in the compounds were identified by comparisons of the resultant IR spectra before and after ozonation. This technique also was deployed for studying various stretching and bending vibrations of the aromatic functional

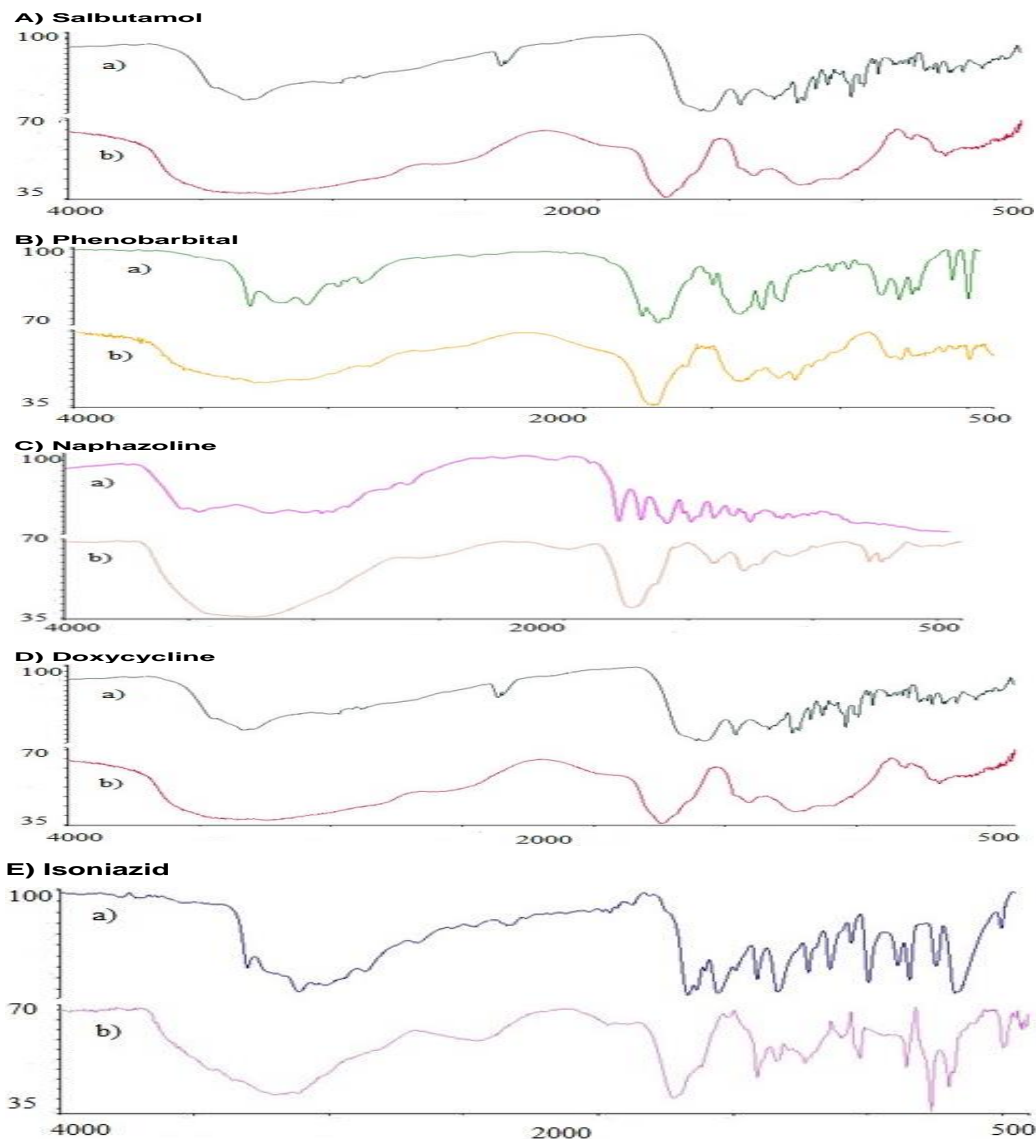


Figure 2. IR spectra of different drug molecules. (a) Before ozonation, (b) After ozonation.

groups. UV/Vis absorption spectra were recorded with a Shimadzu 160A UV/Vis spectrometer. A 1 cm quartz cuvette was used for measuring absorbance and deionized water was used as a blank. The Nuclear Magnetic Resonance (NMR) spectra were recorded in D<sub>2</sub>O using Bruker Drx-500 Avance spectrometers at 296 K. Approximately 50 mg of each dried sample were added to 0.5 ml D<sub>2</sub>O in a 10 mm NMR tube. The signal for D<sub>2</sub>O was used as a reference and set to 4.8 ppm chemical shift. Chemical shifts are given in parts per million ( $\delta$ -scale).

## RESULTS AND DISCUSSION

### Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the compounds were prepared, before and after ozonation. All the samples (Figure 2)

showed band shifting and reductions in the functional groups of the aromatic rings. For comparison the vibration of the O-H bond in hydroxyl groups in the region of  $\sim 3200$  to  $3500\text{ cm}^{-1}$  after ozonation for all compounds are shifted to lower frequencies at  $\sim 3076$  to  $3291\text{ cm}^{-1}$ . This may imply that more OH groups are involved in intermolecular bonds (Allen et al., 2001). In all compounds except naphazoline, there is a broad carbonyl peak in aromatic rings at  $\sim 1730\text{ cm}^{-1}$  due to lactone, followed by a maximum at  $\sim 1739\text{ cm}^{-1}$  from the formation of aliphatic esters. In phenobarbital the peak splits to one additional data at  $1752\text{ cm}^{-1}$  that is related to ketones. In doxycycline and isoniazid, the main peak in the region of  $\sim 1620\text{ cm}^{-1}$  is related to carbonyl groups which disappear after ozonation. This suggests that the carbonyl group is

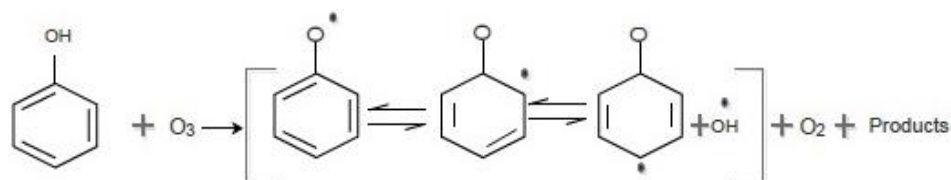


Figure 3. Ozonation process.

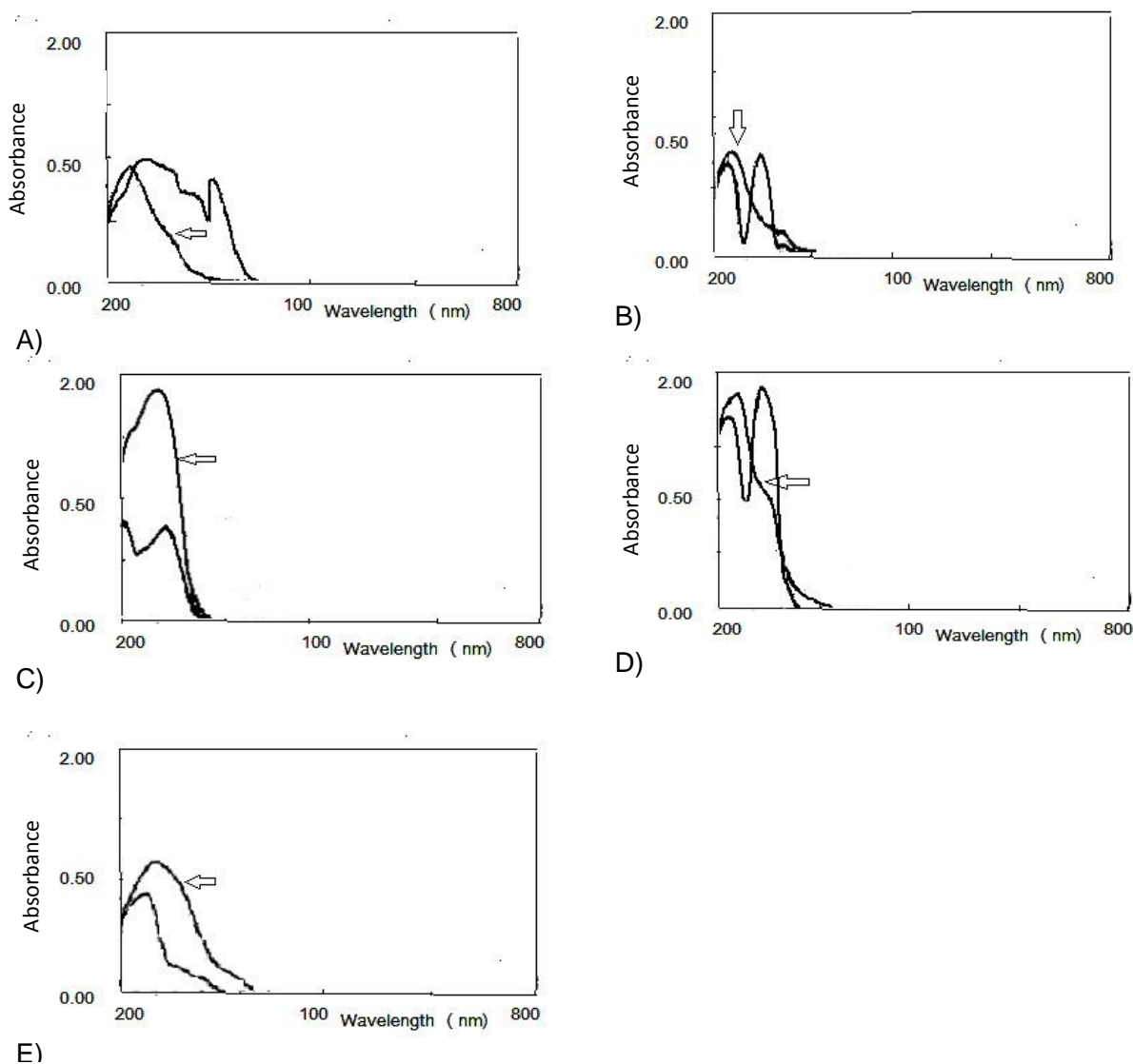


Figure 4. UV-Vis spectra of: (A) Doxycycline, (B) Salbutamol, (C) Isoniazid, (D) Naphazoline and (E) Phenobarbital before and after ozonation.

changed to  $\alpha, \beta$ -unsaturated aliphatic groups (Sahu, 2011).

The appearance of two absorption bands in phenobarbital and isoniazide at  $\sim 1599$  to  $1523\text{ cm}^{-1}$  are consistent with the formation of an unusual but novel  $\text{-C=C-}$  stretch of an enol tautomer of  $\alpha, \beta$ -diketone

product. The band at  $\sim 1407\text{ cm}^{-1}$  is synonymous with the formation of methyl ketones or aldehydes. The bands that appeared at  $\sim 1174\text{ cm}^{-1}$  are related with a mixture of aliphatic esters and ethers. The two shoulders at  $1050$  to  $1000\text{ cm}^{-1}$  typically are due to residual ozonides. Mono substituted terminal vinyl groups also appear to be

formed at  $\sim 900\text{ cm}^{-1}$ . FTIR bands decreasing in phenobarbital and doxycycline are the aromatic in-chain di and tri-substituted vinyl groups absorbing at  $\sim 810\text{ cm}^{-1}$  (Allen et al., 2001).

Some peaks rose due to the treatment of the residue with ozone. In other words, a number of functional groups were apparent in the ozone-treated samples that were elusive before treatment. The application of small doses of ozone may result in polymerization processes (Amy, 1988). It is seen that all the spectra are quite similar. Specifically, the O-H in-plane bending vibration is observed in the region  $1398\text{ to }1413\text{ cm}^{-1}$ . The bands centered in the region  $\sim 2970\text{ to }2872\text{ cm}^{-1}$  in naphazoline, phenobarbital, salbutamol correspond to the stretching mode of asymmetrical  $\text{CH}_3$  and  $\text{CH}_2$  vibrations mainly of aliphatic carbonyl-containing functional groups. The peak centered at  $2794\text{ cm}^{-1}$  in salbutamol may correspond to the stretching mode of symmetrical C-H vibrations of cyclic ether. The absorption peaks detected in the region of  $1500\text{ to }1800\text{ cm}^{-1}$  are typical for the overlapping of the (C=C) stretching vibration mode of the aromatic ring, quinone, with the (C=O) absorption peaks of free carboxylic, ester and carbonyl groups. The most remarkable feature of the spectra is the appearance of a broad carbonyl (C=O) peak in the region  $1709\text{ to }1712\text{ cm}^{-1}$  which is asymmetrical to higher frequencies, and displays a shoulder at  $1770\text{ cm}^{-1}$ .

### Ultraviolet spectrometry (UV visible)

A further literature search on reaction mechanisms shows that most authors are in agreement that the first step consists in hydroxylation of the aromatic ring. The absorption bands for phenol and quinone, and the yields resulting from ring opening, are strong and separate. Some organic impurities also have absorption at 200 to 300 nm. At the same time, there was a split parallel increase in the intensity of absorption at 230 to 240 nm region of the spectrum associated with absorption of the unsaturated carbonyl and carboxylic acids formed in aromatic ring opening. The spectrum of quinone in aqueous solution shows a maximum at 260 nm. The fact that there was no obvious increase in the band at 260 nm ruled out the possibility of the formation of quinone as an intermediate in the interaction of  $\text{O}_3$  with aromatic ring in aqueous solution. The ozone attacks the O-H bond of the aromatic, giving a mixture of several tautomeric radicals which then break down to form the mixed reaction yields (Razumovskii et al., 1979).

A study of the change in UV spectra after ozonation, suggests the progressive disappearance of absorbance maxima which corresponds to bands of aromatic rings (transition  $\pi \rightarrow \pi^*$ ). As for salbutamol, appearance and Disappearance of a band was observed and the absorbance maximum was 245 nm. This band

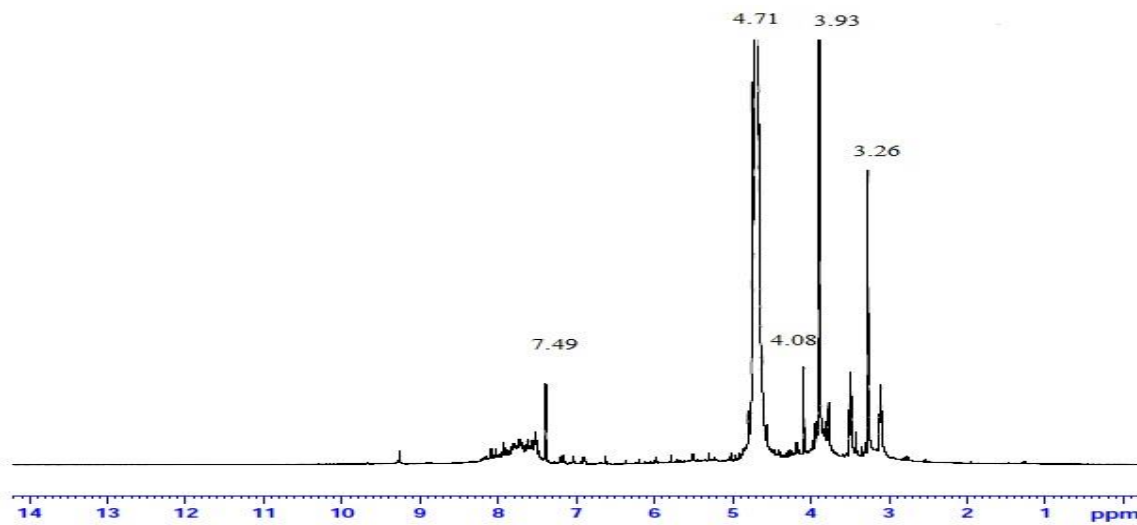
corresponds to the formation of *p*-quinone.

### Spectroscopy

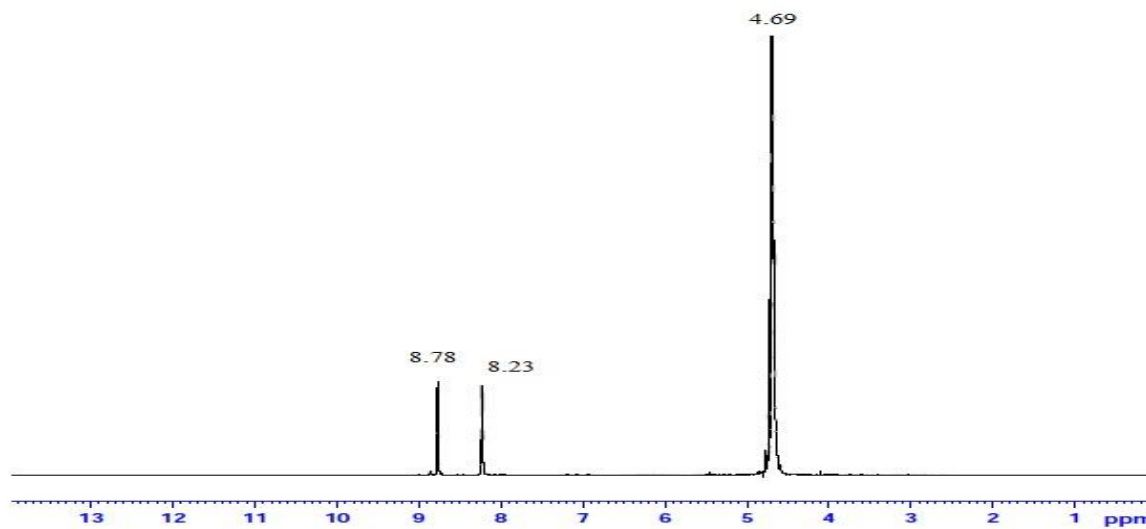
The  $^1\text{H}$ NMR spectrum for all samples confirmed the presence of seven strong proton signals (1.26, 4.08, 4.69, 4.71, 2.56, 3.93 and 3.26 ppm) (Figure 5). The signals at 5.85 and 6.02 ppm are attributable to aromatic hydrogen, respectively. The twin signals at 4.08 and 4.71 ppm for salbutamol and another signal at 4.69 ppm for phenobarbital indicated the presence of three amino groups (NH). These findings are in complete agreement with data obtained from literature reviews (Fotouhi et al., 2006). A small signal between 8.00 and 9.00 ppm for isoniazid is assigned to amide proton. This cleavage seems to be favored due to the possible resonance stabilized carbanion formed from deprotonation of methine carbon. In doxycycline and salbutamol at 0 to 1.6 ppm, protons on methyl and methylene carbons directly bonded to other carbons. The small signal at 1.6 ppm in salbutamol is due to protons on methyl and methylene carbons in alpha position to aromatic rings and the 3.2 ppm signal in phenobarbital, naphazoline and doxycycline is related to carboxyl and carbonyl groups. At 3.2 to 4.3 ppm, protons on methyl, methylene or methine carbons directly bond to oxygen or nitrogen, including carbohydrate and amino acid protons. The small signals at 8.07, 7.49 and 8.23 ppm in doxycycline, naphazoline and isoniazid are assumed to be from protons attached to unsaturated carbons or aromatic protons (Kim and Yu, 2007). Similar signals in doxycycline and phenobarbital were observed at 0.9 to 1.3 ppm, assigned mainly to saturated  $\text{CH}_2$  groups. Within the region of 2.8 to 4.4 ppm, an increased signal was noticed, which indicates an increase in oxygen-containing functional groups. The signal at 3.07 ppm in doxycycline and salbutamol is due to H and  $\text{CH}_3$  groups and the 3.93 ppm in naphazoline and salbutamol is for  $\text{CH}_2$ ; it implies the 6.82 to 7.74 CH aromatic disappeared. In addition, the 4.65 ppm for all samples is related to  $\text{CH}_2$  (Grinhut et al., 2011, Ohlenbusch et al., 1998). Thus, the obtained  $^1\text{H}$ NMR data obtained support the hypothesis that the ozonation of aromatic compounds can be the result of cleavage of the aromatic rings. Comparatively, the protons of the products are much more than aromatics counterparts. However, comparison of the NMR and optical data provides evidence for a complex mechanism of interactions of substrates with ozone, and does allow us to state with certainty the cleavage of aromaticity direct coordination.

### Conclusions

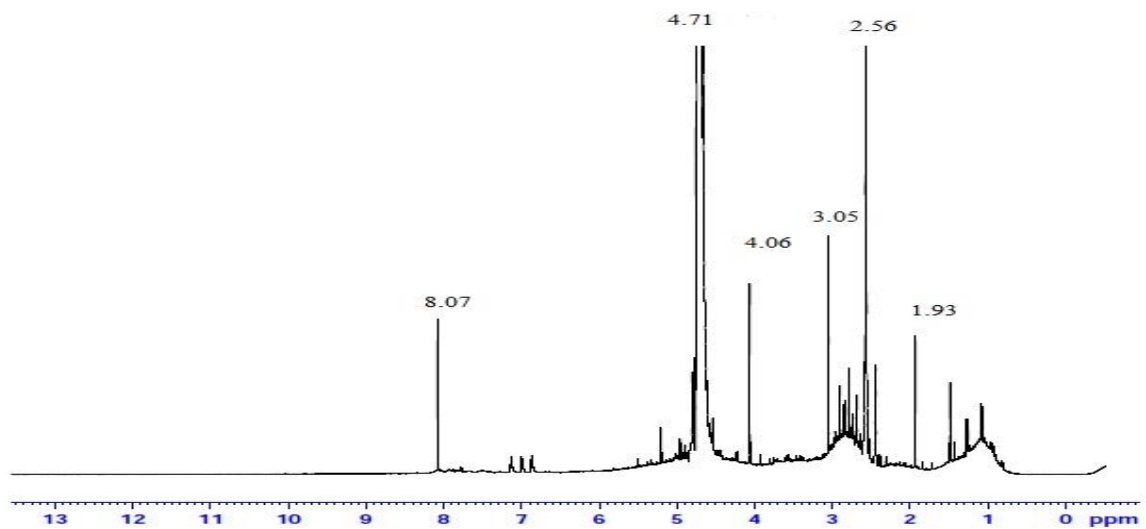
The data demonstrates exposure of some drugs with aromatic rings to ozone gas resulted in the consistent



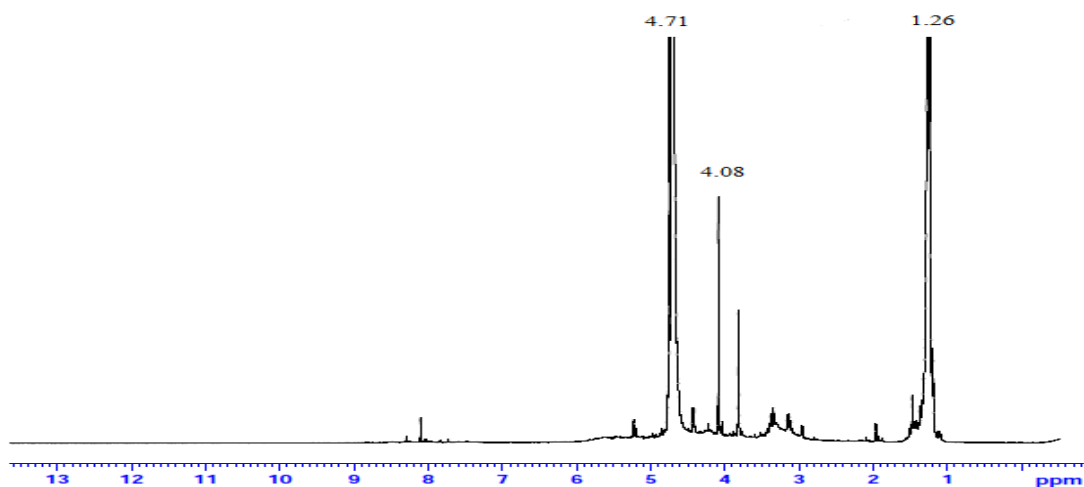
A)



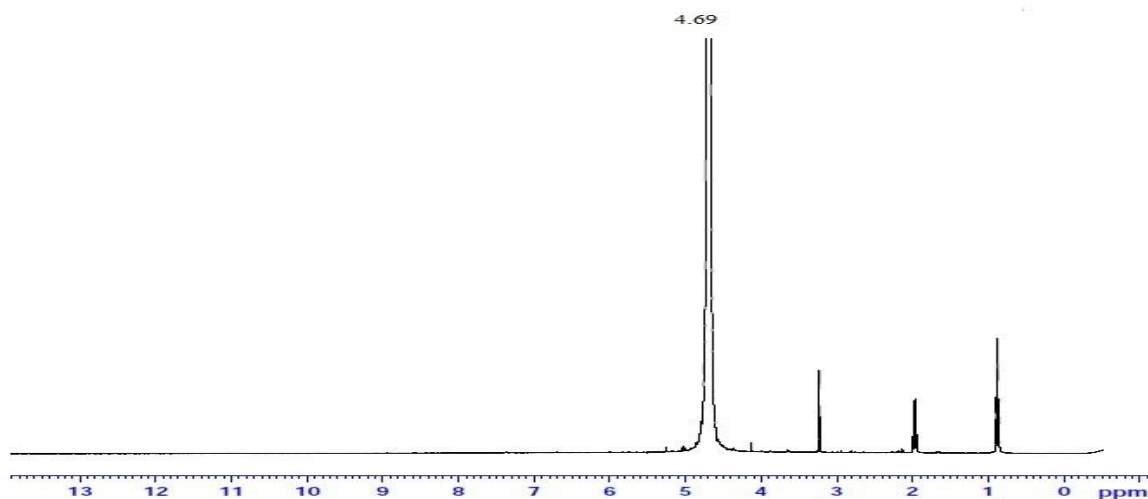
B)



C)



D)



E)

**Figure 5.** NMR spectra of: (A) Naphazoline, (B) Isoniazid, (C) Doxycycline, (D) Salbutamol and (E) Phenobarbital after ozonation.

formation of unsaturated yields based on aliphatic esters, ketones, and lactones as well as aromatic cleavage. Results obtained during the ozonation of aqueous solutions of aromatic compounds have shown that the reaction occurs in two steps: A first phase leading to the opening of the aromatic ring and a second phase in which the sub-products, resulting from the first phase, are stable towards further ozonation. They do not, consequently, consume ozone; moreover they are biodegradable (Evison, 1977; Hann, 1956). Since ozone is such a powerful oxidizer, this suggests this process may be used for the destruction of organic substances. It is evident that ozone preferably oxidizes in electron-rich

sites (Hoighe and Bader, 1983). As a result, a dramatic decrease in UV absorbance at 254 to 280 nm is observed during ozonation of the aromatic compounds. It is known that during ozonation in water solutions, hydroxyl radicals ( $\text{OH}\cdot$ ) proceed along with direct ozonation.  $\text{OH}\cdot$  radicals react relatively unselectively (Hoighe, 1982; Staehelin and Hoigne, 1985). In its turn,  $\text{OH}\cdot$  radicals produce organic radicals as a result of their reactions with organic compounds. The reactions with  $\text{OH}\cdot$  radicals may bring about considerable structural changes in macromolecules including hydroxylation, decarboxylation, and depolymerisation of the initial materials, producing oxidized structures that are hydrophobic and less aromatic.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENT

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