Research Article

Kinetics and mechanism of the oxidative degradation of sofosbuvir by *N*-bromosuccinimide in aqueous medium

Alaa Eldin Mokhtar Abdel-Hady[1] · **Ashraf M. Taha**[1]

Abstract
The kinetics of the electron transfer in sofosbuvir (Sofo) using *N*-bromosuccinimide (NBS) in aqueous medium has been studied spectrophotometrically over the (10–30) °C range, (0.1–0.5) mol dm^{-3} ionic strength, pH = 6.0–8.0 range over a range of NBS and Sofo concentrations. The rate of reaction was first order dependence on both [NBS] and [Sofo] and decreased with increasing $[H^+]$ over the range studied suggesting that the deprotonated form of sofosbuvir is more reactive than its conjugate acid. The hydrogen atom attached to the nitrogen atom of pyrimidine dione ring has a highly acidic character due to withdrawing effect of the adjacent two carbonyl groups and so the sofosbuvir acted as an acid. The rate decreased with increasing the ionic strength of the reaction mixture and supported that the reaction took place between two ions of different charges.

Keywords Sofosbuvir · *N*-bromosuccinimide · Kinetics of oxidation · Inner-sphere mechanism

1 Introduction

Sofosbuvir, (Sofo) is an antiviral agent against hepatitis C virus [1]. It was reported by the World Health Organization (WHO) that hepatitis C is considered as a global health problem, and more than 3% of the world's peoples were infected with hepatitis C virus. According to the released Egyptian Demographic Health Survey (EDHS), Egypt has the largest epidemic of HCV in the world and the overall percentage of Egyptian peoples that have positive results for hepatitis C antibody was 14% [2]. The drug showed degradation under oxidative, photolysis, acid and base hydrolysis conditions. Oxidative degradation of sofosbuvir was carried out by 3% H_2O_2 and 6% H_2O_2 at room temperature for 10 days [3]. It was found that 3% H_2O_2 was ineffective to oxidize the drug even after 10 days, whereas 6% H_2O_2 lead to 11% degradation of the drug. Since the drug is recently introduced to the market, no much literature has been reported for its degradation or its kinetics of oxidation by different oxidizing agents.

N-Bromosuccinimide (NBS) is an organic compound that is used for oxidation and selective bromination of organic compounds and substrates on a large scale [4–6]. Three different oxidation pathways using NBS could be observed including the coordination to the substrate through the carbonyl group [7], homolytic dissociation of N-Br bond to give bromine radical which acts as an oxidizing agent or heterolytic dissociation to give bromium ion (Br^+) which also acts as an oxidizing agent [8–12]. Oxidation of gabapentin (GBP) with NBS was studied under varied conditions [13]. The kinetic reaction was first order dependence on [GBP], fractional order on $[H^+]$ and zero order dependence on [NBS]. It was surprising that the reaction rate decreased with temperature over the range (25–40) °C. This behavior has been ascribed to a highly exothermic rate determining step. Oxidative degradation of rhodamine-B (RhB) with NBS in aqueous and different water alcohol solvent mixtures has been investigated over varied conditions of [RhB], [NBS], pH and temperatures [14]. The reaction exhibited a first-order dependence on

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[NBS], [RhB], inverse first-order dependence on [OH^-] and decreased with decreasing the dielectric constant of the medium. Also, the oxidation of L-proline by NBS in aqueous media was carried out [15]. The reaction rate was first-order dependence on [NBS], [L-proline] and decreased with increasing [H^+] over (2.6–3.3) pH range.

In the present work, there was an intention to study the kinetics and mechanism of the electrons transfer in sofosbuvir (Sofo) by *N*-bromosuccinimide (NBS), since the drug has been recently discovered and no much literature has been reported for its degradation or kinetics of oxidation by different oxidizing agents.

2 Experimental

2.1 Materials and methods

BDH, Fluka or Analar chemicals were used in this study without any further purifications. Sofosbuvir was obtained as a gift sample from Pharco B International (Chemical), Egypt and the working solution was prepared in doubly distilled water. This working solution was stable for 2 weeks at room temperature. Freshly prepared solutions of *N*-bromosuccinimide (NBS) were prepared daily. The pH of the reaction medium was maintained constant using K_2HPO_4 and citric acid buffer. Aqueous solution of NaCl of known concentration has been used to change the ionic strength of the reaction mixture. Bidistilled water has been used in all kinetics run and preparations.

2.2 Kinetic procedure

A Shimadzu 1700UV-visible spectrophotometer was used to follow the rate of oxidation reaction. The initial rates of oxidation were followed by measuring the decrease in the absorbance of sofosbuvir at its characteristic wavelength, ($\lambda = 260$ nm) for a definite period of time, Fig. 1. The reactants, other than NBS were mixed and equilibrated at the required temperature for 15–20 min. The required volume of separately thermostated NBS stock solution was rapidly added to the reaction mixture and a sample was transferred to the measuring cell where the absorbance was measured for a definite period of time. The pH of the reaction was determined using 3505 Jenway pH-meter. Pseudo-first order conditions were applied in all kinetic runs using excess (at least tenfold) concentrations of NBS as compared to the concentrations of sofosbuvir.

The stoichiometry of the reaction was determined by using different [NBS]:[Sofo] ratios, keeping the concentration of (Sofo) at least twice over that of [NBS] and the mixtures were kept a side for 24 h. The concentration of the unreacted (Sofo) was measured by dividing the absorbance at $\lambda = 260$ nm by its molar absorptivity. The experimental results showed that 1 mol of (Sofo) consumed 1 mol of NBS and consistent with Eq. 1

$$[\text{Sofo}]_T + [\text{NBS}] \rightarrow \text{products} \quad (1)$$

where $[\text{Sofo}]_T$ represented the total concentration of all sofosbuvir species in the solution

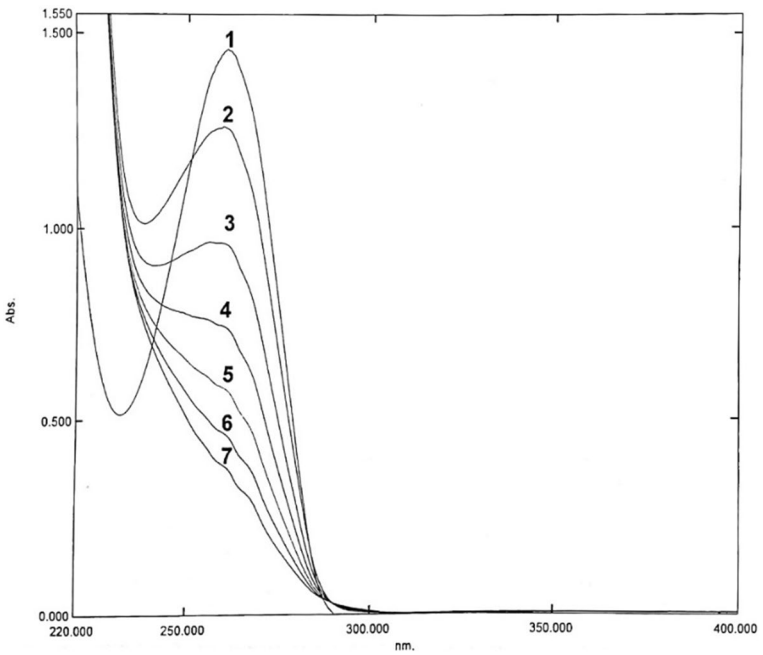


Fig. 1 Absorption spectra of the reaction mixture at [Sofo] = 5×10^{-5} mol dm^{-3}, [NBS] = 0.56×10^{-3}mol dm^{-3}, pH = 7.6, and I = 0.05 mol dm^{-3}. Peaks 1, 2, 3, 4, 5, 6 and 7 were taken at 0, 60, 90, 120, 180, 240 and 300 s from the time of initiation of reaction

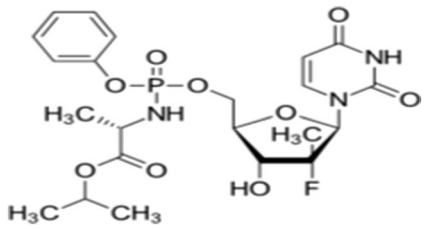


Fig. 2 Structure of sofosbuvir

3 Results and discussion

Sofosbuvir is a monophosphorylatedpyrimidine with chemical formula as shown in Fig. 2.

Figure 2 Chemical structure of sofosbuvir ($C_{22}H_{29}FN_3O_9P$, M.wt = 529.453). Isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxyphosphorryl] amino] propanoat.

Sofosbuvir was approved to be used alone or in combination with ribavarin and ledipasvir for the treatment of HCV [16, 17]. The hydrogen atom attached to the nitrogen of pyrimidine dione ring has a highly acidic character due to withdrawing effect of the adjacent two carbonyl groups and so the sofosbuvir acted as an acid.

Oxidation of sofosbuvir by NBS was studied over (6.0–8.0) pH range, ionic strength (0.10–0.50) mol dm^{-3} and (10–30) °C using different [Sofo] and [NBS]. The rate of oxidation reaction was measured at fixed NBS concentration, ionic strength, pH and temperature. Plots of (A_t) versus time, where A_t was the absorbance of the drug at time t, were curved indicating that the reaction was not zero order dependent on sofosbuvir. Variation of $\ln(A_\infty - A_t)$,where A_∞ and A_t were the absorbance of sofosbuvir at infinity and at time t respectively versus time was linear up to ≥ 80% of the reaction indicating that the reaction was first order. The pseudo-first order rate constant, k_{obs} was calculated from the slopes of the first order plots.

3.1 Effect of [Sofo] concentration on k_{obs}

The effect of [Sofo] concentrations on k_{obs} was studied over the range $(5.0–6.4) \times 10^{-5}$ mol dm^{-3} and keeping other parameters at constant values. Kinetics data Table 1 and Fig. 3 indicated that the values of k_{obs} were unchanged when the concentration of (Sofo) was varied at constant [NBS], proving that the reaction was first order dependent on [Sofo]. Furthermore, plot of log initial rate (− d[Sofo]/dt) versus log initial [Sofo] was linear with slope = 1.0 ± 0.15. The reaction rate can therefore be represented by Eq. 2

$$\left(-d\left[\text{Sofo}\right]/dt\right) = k_{obs}\left[\text{Sofo}\right]_T \quad (2)$$

Table 1 Variation of the reaction rate on [Sofo], [NBS] and temperatures at pH = 7. 80, I = 0.05 mol dm^{-3}

T (°C)	10^5 [Sofo] (mol dm^{-3})	10^3 [NBS] (mol dm^{-3})	10^3 k_{obs} (s^{-1})
10	5.00	0.56	4.08
10	5.00	0.90	4.94
10	5.00	1.10	5.29
10	5.00	1.30	5.49
10	5.00	1.70	5.99
10	5.00	2.20	6.33
10	5.00	2.80	6.50
10	5.00	3.06	6.66
10	5.00	3.33	6.71
10	5.20	0.90	4.83
10	5.40	0.90	4.81
10	5.60	0.90	4.78
10	5.80	0.90	4.75
10	6.00	0.90	4.80
10	6.40	0.90	4.80
15	5.00	0.56	4.81
15	5.00	0.90	5.82
15	5.00	1.10	6.05
15	5.00	1.30	6.54
15	5.00	1.70	6.93
15	5.00	2.20	7.30
15	5.00	2.80	7.41
15	5.00	3.06	7.81
15	5.00	3.33	7.69
20	5.00	0.56	5.85
20	5.00	0.90	6.94
20	5.00	1.10	7.35
20	5.00	1.30	7.69
20	5.00	1.70	8.19
20	5.00	2.20	8.48
20	5.00	2.80	8.69
20	5.00	3.06	8.82
20	5.00	3.33	8.93
30	5.00	0.56	6.78
30	5.00	0.90	7.91
30	5.00	1.10	8.37
30	5.00	1.30	8.73
30	5.00	1.70	9.14
30	5.00	2.20	9.43
30	5.00	2.80	9.80
30	5.00	3.06	9.98
30	5.00	3.33	10.10
35	5.00	0.56	8.19
35	5.00	0.90	9.39
35	5.00	1.10	9.82
35	5.00	1.30	10.22
35	5.00	1.70	10.75
35	5.00	2.20	10.99

Table 1 (continued)

T (°C)	10^5 [Sofo] (mol dm^{-3})	10^3 [NBS] (mol dm^{-3})	10^3 k_{obs} (s^{-1})
35	5.00	2.80	11.49
35	5.00	3.06	11.36
35	5.00	3.33	11.52

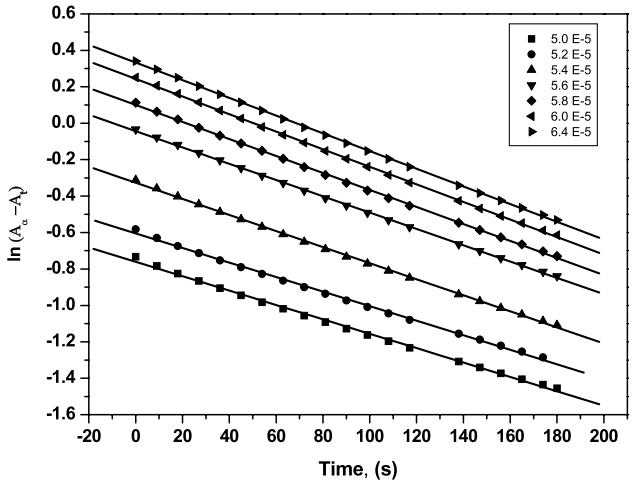


Fig. 3 Plots of $\ln(A_\infty - A_t)$ versus time at different [Sofo]. [NBS] = 0.56×10^{-3} mol dm^{-3}, pH = 7.6, I = 0.05 mol dm^{-3} and T = 25 °C

where $[Sofo]_T$, represented the total [Sofo] present. k_{obs} including the value of the specific rate constant and the concentration of NBS.

3.2 Effect of temperatures on k_{obs}

The effect of temperature on the rate of oxidation was studied over the range (10–30) °C and keeping all other parameters constant. The experimental data Table 1 indicated that the values of k_{obs} increased with temperature over the range studied. At constant [H^+] and ionic strength, $1/k_{obs}$ varied linearly with 1/[NBS] at different temperatures Fig. 4 according to the equation $1/k_{obs} = m(1/[NBS] + C$ with correlation coefficients $r_{10} = 0.99945$, $r_{15} = 0.99729$, $r_{20} = 0.99943$, $r_{30} = 0.99928$ and $r_{35} = 0.99878$ Where r_{10}, r_{15}, r_{20}, r_{30} and r_{35} were the correlation coefficients at 10,15, 20,30 and 35 °C respectively. So, the relation between k_{obs} and [NBS] at different temperatures was represented by Eq. 3

$$k_{obs} = \{a[NBS]/1 + b[NBS]\} \quad (3)$$

and

$$1/k_{obs} = 1/a[NBS] + b/a \quad (4)$$

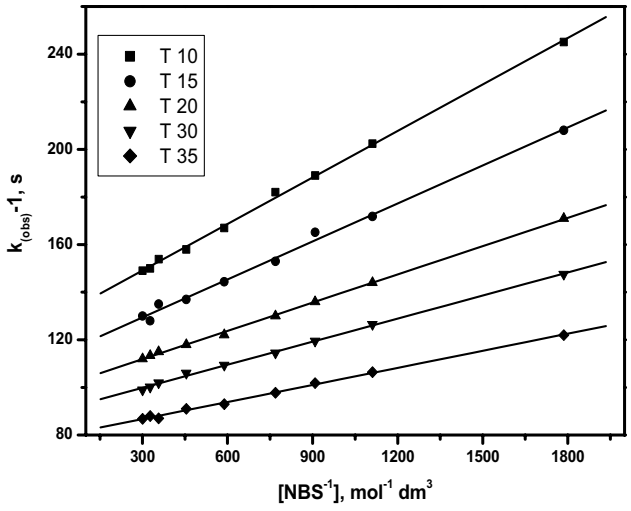


Fig. 4 Plots of $1/k_{obs}$ versus 1/[NBS] at different temperatures

3.3 Effect of pH on k_{obs}

The effect of pH on the reaction rate was studied by varying the pH values using citric acid and KH_2PO_4 buffer over the range (6.0–8.0) at constant [NBS], [Sofo], ionic strength and temperatures. Data in Table 2 indicated that the rate of the electrons transfer reaction was increased by decreasing [H^+] over the pH range considered and supported the participation of the deprotonated form of sofosbuvir in the slowest steps.

Plots of $1/k_{obs}$ versus 1/[NBS] at different pHs (6.0–8.0) Fig. 5 indicated that the rate of reaction increased with increasing pH, Table 2 and fits the linear relation, $1/k_{obs} = m(1/[NBS]) + C$ with correlation coefficients of 0.9970, 0.9910, 0.9979, 0.9973, and 0.9773, at pHs, 6.0, 6.4, 6.8, 7.6, and 8.0 respectively.

3.4 Effect of ionic strength

Effect of ionic strength on the rate of oxidation was examined using different values of ionic strength for the reaction mixture and keeping other parameters constant. The experimental data Table 3 showed that the reaction rate decreased by increasing the ionic strength and proved that the reaction took place between ions of different charges. So the main reactive ionic species are the anion of sofosbuvir and the bromium ion (Br^+) which produced by heterolytic dissociation of N–Br bond.

3.5 Identification of the oxidation products

The oxidative degradation products of sofosbuvir in addition to acid, base degradation impurity were reported [18]. Oxidative degradation, acid and base chromatograms

Table 2 Effect of pH on k_{obs} at [Sofo] = 5.0×10^{-5} mol dm^{-3}, I = 0.05 mol dm^{-3} and T = 10 °C

10^3 [NBS], (mol dm^{-3})	10^3 k_{obs} (s^{-1})								
pH	0.56	0.90	1.10	1.30	1.70	2.20	2.80	3.06	3.33
6.0	0.85	1.12	1.29	1.45	1.56	1.81	1.84	1.98	1.91
6.4	1.60	1.94	2.24	2.35	2.80	3.05	3.35	3.49	3.61
6.8	2.46	3.08	3.31	3.71	3.99	4.33	4.52	4.56	4.75
7.6	3.04	3.84	4.37	4.67	4.92	5.51	5.90	6.20	6.27
8.0	4.46	5.67	6.03	6.33	6.91	7.26	6.90	7.04	7.13

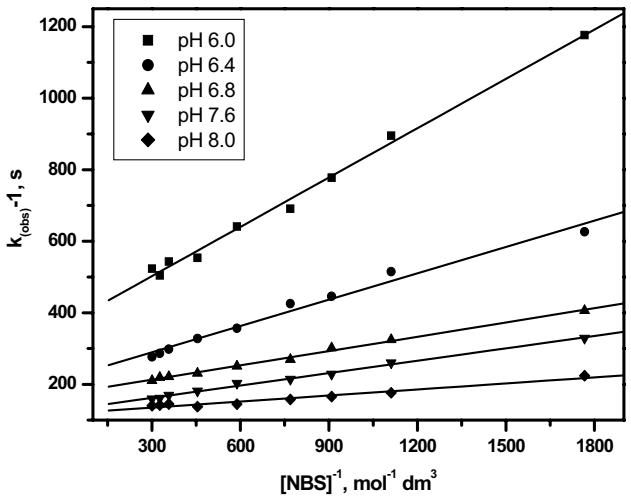


Fig. 5 Plots of $1/k_{obs}$ versus 1/[NBS] at different pH's

Table 3 Effect of ionic strength at [Sofo] = 5.0×10^{-5} mol dm^{-3}, pH = 8.0, [NBS] = 1.10×10^{-3} mol dm^{-3} and T = 10 °C

I (mol dm^{-3})	10^3 k (s^{-1})
0.10	5.84
0.20	5.05
0.30	4.31
0.40	3.71
0.50	3.36

were shown in Figs. 6, 7 and 8. In oxidative degradation study, degradation was observed very less and with obtained quantity (less than 2 mg) recorded 1H NMR and HRMS analysis Figs. 9, 10, 11, 12, 13, 14 and 15 From the spectral data, oxidation degradation product was showing molecular weight of 527.15, molecular formula $C_{22}H_{27}FN_3O_9P$ and its name as (S)-isopropyl 2-((S)-(((2R, 4S, 5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-4-methyl-3 oxotetrahydrofuran2yl) methoxy) (phenoxy) phospho-rylamino)propanoate. The acid oxidation was showing molecular weight of 416.08, molecular formula $C_{16}H_{18}FN_2O_8P$ and its name as (R)-((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydr- ofura-n-2-yl) methylphenyl hydrogen phosphate. In base degradation study two impurities were observed and were isolated. Impurity-A was showing molecular weight of 453.13, molecular formula $C_{16}H_{25}FN_3O_9P$ and its name as (S)-isopropyl 2-((R)-(((2R, 3R, 4R, 5R)-5-(2,4-dioxo-3,4-dihydro pyramid-in-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(hydroxyl) phospho -rylamino) propanoate. Isolated base degradation impurity-B was showing molecular weight of 411.08, molecular formula $C_{13}H_{19}FN_3O_9P$ and its name as (S)-2-((R)-(((2R, 3R, 4R, 5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(hydroxy)phosphorylamino) propanoic acid.

3.6 Test for free radicals

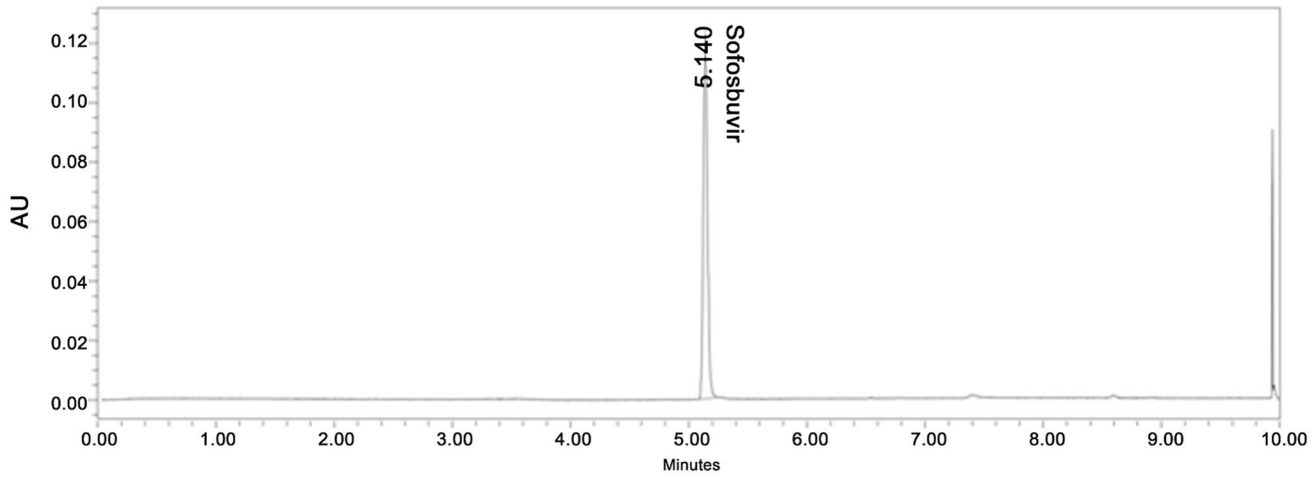
In order to check the presence of the free radicals intermediates in the reaction mixture, the following test was performed. A reaction mixture containing acrylonitrile was kept aside for 24 h at the experimental conditions. On addition of methanol, no precipitate was observed suggesting that no free radical could be detected in the reaction. Addition of $AgNO_3$ to the reaction mixture led to a slow formation of a pale yellow AgBr precipitate. When succinimide (the reduced form of NBS) was added to the reaction mixture, no significant effect on the rate reaction was observed. In aqueous medium the succinimide anion abstract H^+ from the medium to form succinimide rather than dimerize to bisuccinimide [19, 20].

Since the oxidation of organic compounds by NBS can proceed via the bromium ion (Br^+) in polar media. The mechanistic pathway for the oxidation of sofosbuvir by NBS may be interpreted by the following reaction scheme,NBS $\rightleftharpoons$ $Br^+ + R^-$ where R^- is succinimide anion.

$$[\text{Sofo - H}] \overset{K_3}{\rightleftharpoons} [\text{Sofo}]^- + H^+ \quad (5)$$

$$[\text{Sofo - H}] + [Br^+] \overset{K_4}{\rightleftharpoons} [\text{Sofo - H - Br}]^+ \quad (6)$$

$$[\text{Sofo}]^- + [Br^+] \overset{K_5}{\rightleftharpoons} [\text{Sofo - Br}] \quad (7)$$



Standard chromatogram of Sofosbuvir.

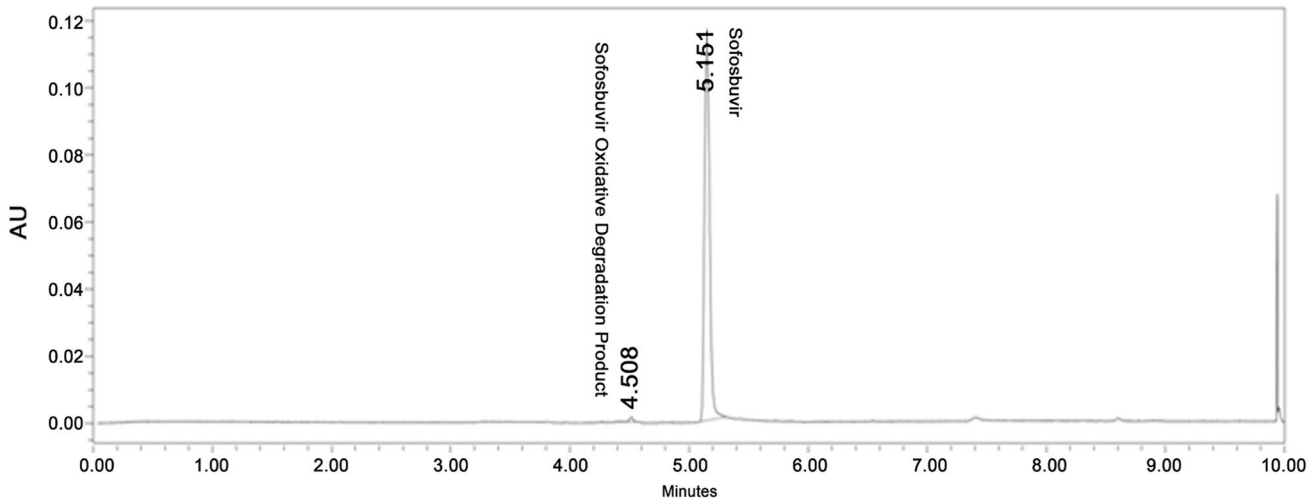


Fig. 6 Oxidative degradation chromatogram of Sofosbuvir

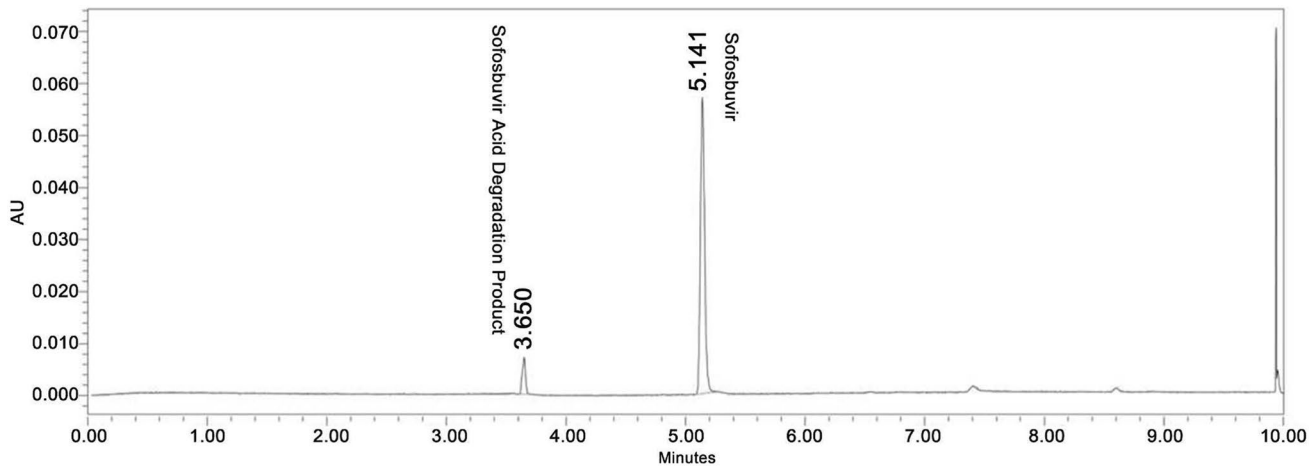


Fig. 7 Acid degradation chromatogram of Sofosbuvir

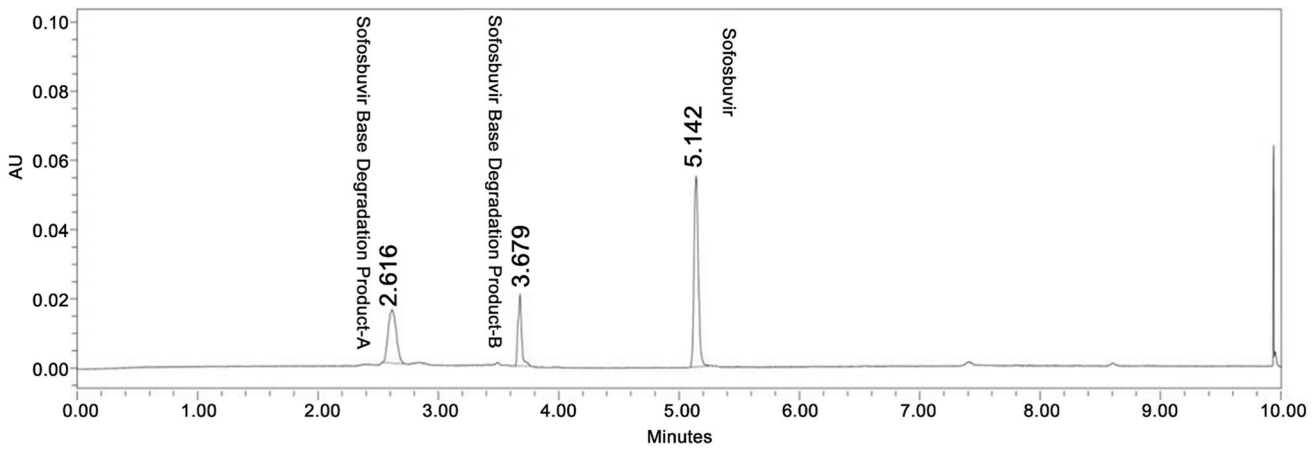


Fig. 8 Base degradation chromatogram of Sofosbuvir

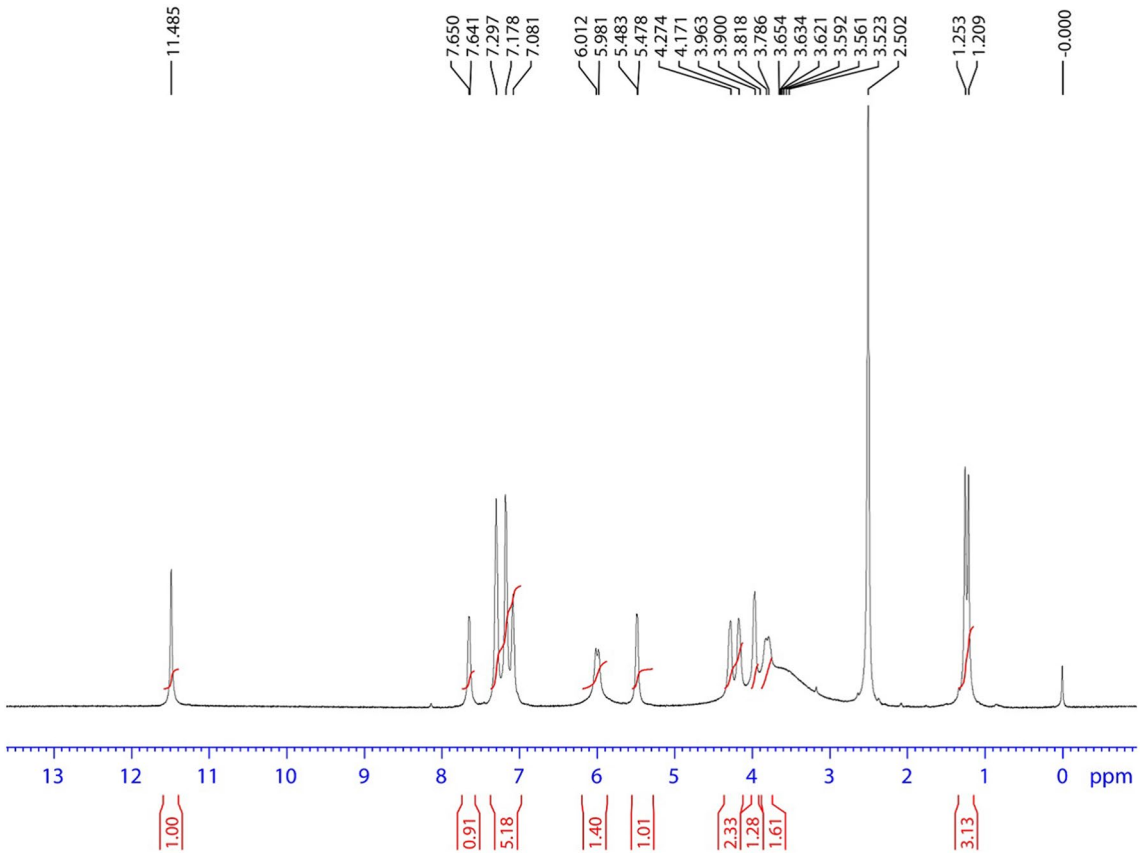


Fig. 9 ^{1}H NMR spectrum of acid degradation product

$$[\text{Sofo - H - Br}]^{+} \xrightarrow{k_1} \text{products} \quad (8)$$

$$[\text{Sofo - Br}] \xrightarrow{k_2} \text{products} \quad (9)$$

From the above mechanism, the rate law of the reaction is represented by,

$$\begin{aligned} \text{rate} &= k_1 \left[\text{Sofo - H - Br}\right]^{+} + k_2 \left[\text{Sofo - Br}\right] \\ &= \left[\text{Sofo - H}\right]\left[Br^{+}\right]\left\{k_1 K_2 + K_3 K_5 k_2 / \left[H^{+}\right]\right\} \end{aligned} \quad (10)$$

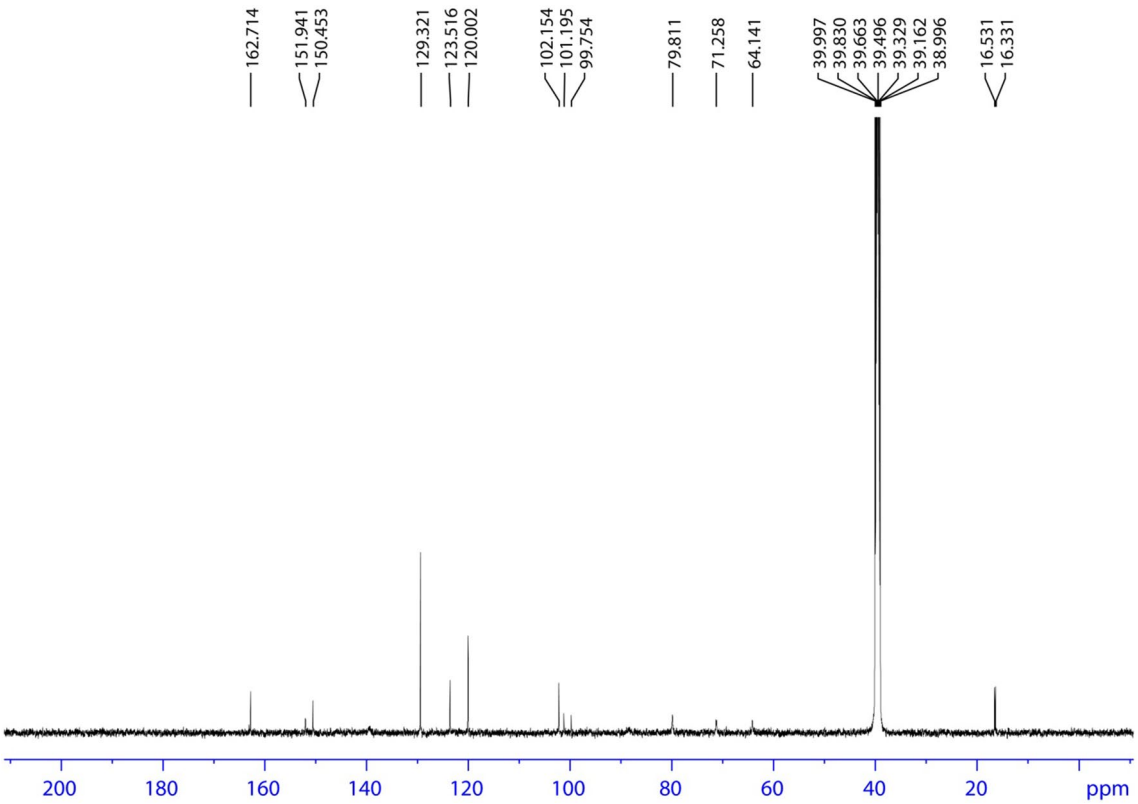


Fig. 10 ^{13}C NMR spectrum of acid degradation product

From the suggested mechanistic pathway, then

$$\begin{aligned}\left[\text{Sofo - H}\right]_T &= \left[\text{Sofo - H}\right]\left\{1 + K_3/\left[H^+\right] + \left[Br^+\right]\left(K_4 + K_3K_5/\left[H^+\right]\right)\right\} \text{ and}\\ \left[\text{Sofo - H}\right] &= \left[\text{Sofo - H}\right]_T/\left\{1 + K_3/\left[H^+\right] + \left[Br^+\right]\left(K_4 + K_3K_5/\left[H^+\right]\right)\right\}\end{aligned} \tag{11}$$

Substitution in Eq. (10),then

$$\text{rate} = \left\{\left[Br^+\right]\left[\text{Sofo - H}\right]_T\left(k_1K_4 + K_3K_5k_2/\left[H^+\right]\right)\right\}/\left\{1 + K_3/\left[H^+\right] + \left[Br^+\right]\left(K_4 + K_3K_5/\left[H^+\right]\right)\right\} \tag{12}$$

Since, the deprotonated form of the complex, [Sofo-Br] is assumed to be more reactive than its conjugate acid, then $k_5 \gg k_4$, and Eq. (12) is reduced to Eq. (13)

$$\text{Rate} = \left[Br^+\right]\left[\text{Sofo - H}\right]_T\left(K_3K_5k_2/\left[H^+\right]\right)/\left\{1 + K_3/\left[H^+\right] + \left[Br^+\right]\left(K_4 + K_3K_5/\left[H^+\right]\right)\right\} \tag{13}$$

$$\begin{aligned}k_{obs} &= \left[Br^+\right]\left(K_3K_5k_2/\left[H^+\right]\right)/\left\{1 + K_3/\left[H^+\right] + \left[Br^+\right]K_4 + K_3K_5/\left[H^+\right]\right\} \text{ and}\\ 1/k_{obs} &= \left[H^+\right] + K_3 + \left[Br^+\right]\left(K_4\left[H^+\right] + K_3K_5\right)/\left[Br^+\right]\left(K_3K_5k_2\right)\\ &= \left[H^+\right] + K_3/\left[Br^+\right]\left(K_3K_5k_2\right) + K_4\left[H^+\right] + K_3K_5/K_3K_5k_2\end{aligned} \tag{14}$$

At constant $[H^+]$, Eq. (14) is consistent to Eq. (4) where.

$a = \left(K_3K_5k_2\right)/\left[H^+\right] + K_3$ and

$b = \left(K_4\left[H^+\right] + K_3K_5\right)/K_3 + \left[H^+\right]$

The graphical relation between 1/a and $[H^+]$ was linear of form, $y = mx + c$ with slope $= 3.84 \times 10^5$ and intercept $= 0.0797$. K_3 was calculated by dividing the intercept by the slope of the plot as 2.07×10^{-7} mol dm^{-3}. Also, b/a varies linearly with $[H^+]$ according to the equation $y = mx + c$ with slope $= 2.42 \times 10^8$ and intercept $= 122.66$.

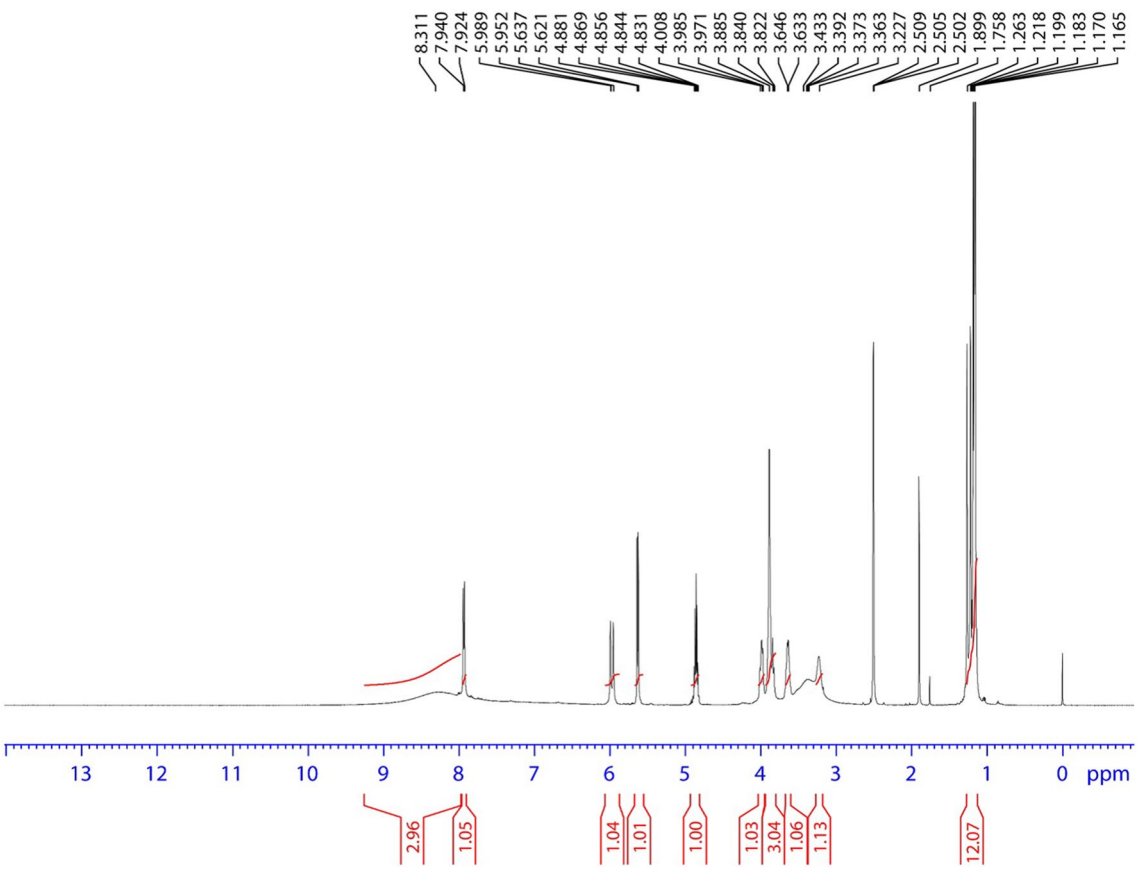


Fig. 11 ^{1}H NMR spectrum of base degradation product-A

The specific rate constant k_2 was measured from the reciprocal of the intercept as 8.15×10^{-3} mol^{-1} dm^3 s^{-1}. Value of K_5 was calculated from quotient of the intercept of b/a versus [H$^+$] by the intercept of 1/a versus [H$^+$] as 1.54×10^3 mol^{-1} dm^3. Replacement of the values of K_3, K_5 and k_2, K_4 can be calculated from the slope of b/a versus [H$^+$] as 628.73. The thermodynamic parameters ($\Delta H^{\ddagger}$) and ($\Delta S^{\ddagger}$) accompanied to the factor a Table 4 were measured by least square fit to the transition state theory equation as 24.8 kJ mol^{-1} and − 134.1 J K^{-1} mol^{-1} respectively. $\Delta H^{\ddagger}$ is a composite value including the enthalpy of formation accompanied with the intermediate complex, [Sof-Br] and the enthalpy of activation of the intramolecular electron transfer step. The positive $\Delta H^{\ddagger}$ value indicated that the formation of the precursor intermediate complex is an endothermic process. The $\Delta S^{\ddagger}$ negative value was ascribed to increase the result of mutual ordering of the solvated water molecules of the equilibrium and intramolecular electron transfer step.

An inner-sphere mechanism was considered for the oxidation of both protonated and deprotonated forms of sofosbuvir. The deprotonated form was considered as more reactive than its conjugate acid. Formation of Br^- as a product to the oxidation process was detected by precipitation of AgBr upon addition of $AgNO_3$ solution to the reaction mixture

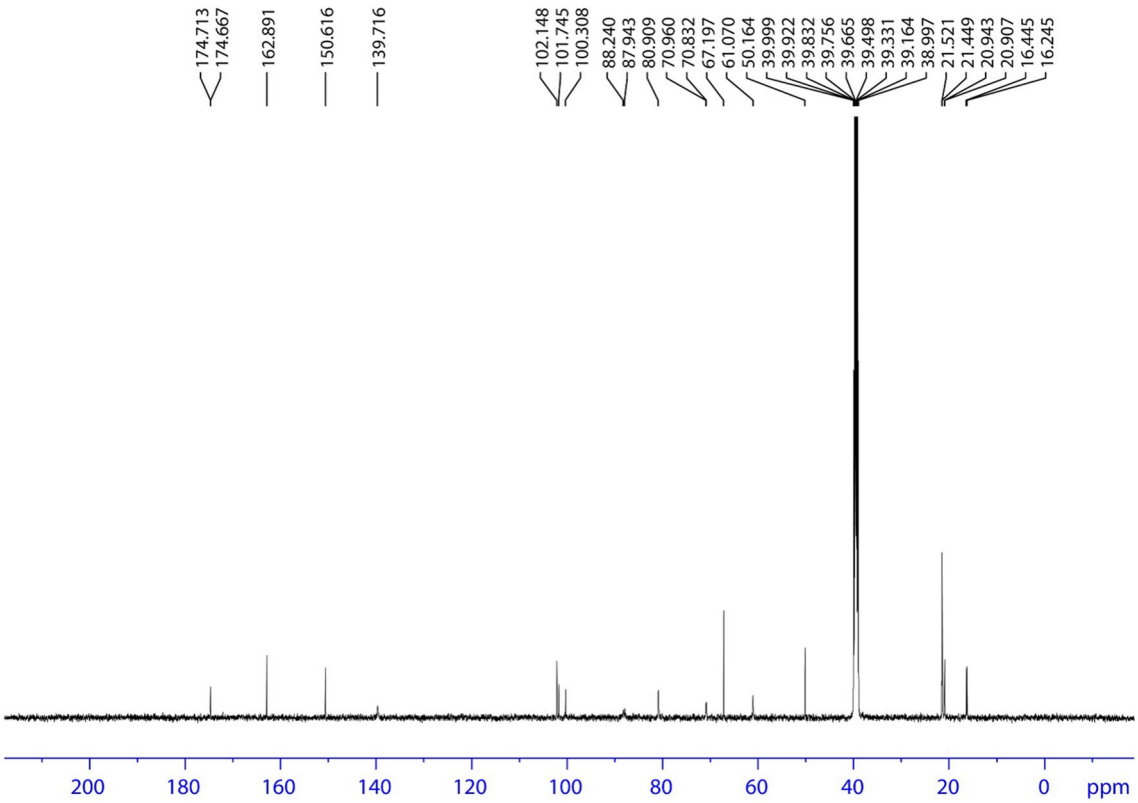


Fig. 12 ^{13}C NMR spectrum of base degradation product-A

4 Conclusion

Sofosbuvir acted as an acid. The hydrogen atom attached to the nitrogen atom of pyrimidine dione ring has a highly acidic character due to withdrawing effect of the adjacent two carbonyl groups. The rate of oxidation was increased by decreasing $[H^+]$ of the reaction mixture and supported that the deprotonated form of sofosbuvir was more reactive than its conjugate acid. The reaction rate decreased with increasing the ionic strength and proved that the reaction took place between ions of different charges.

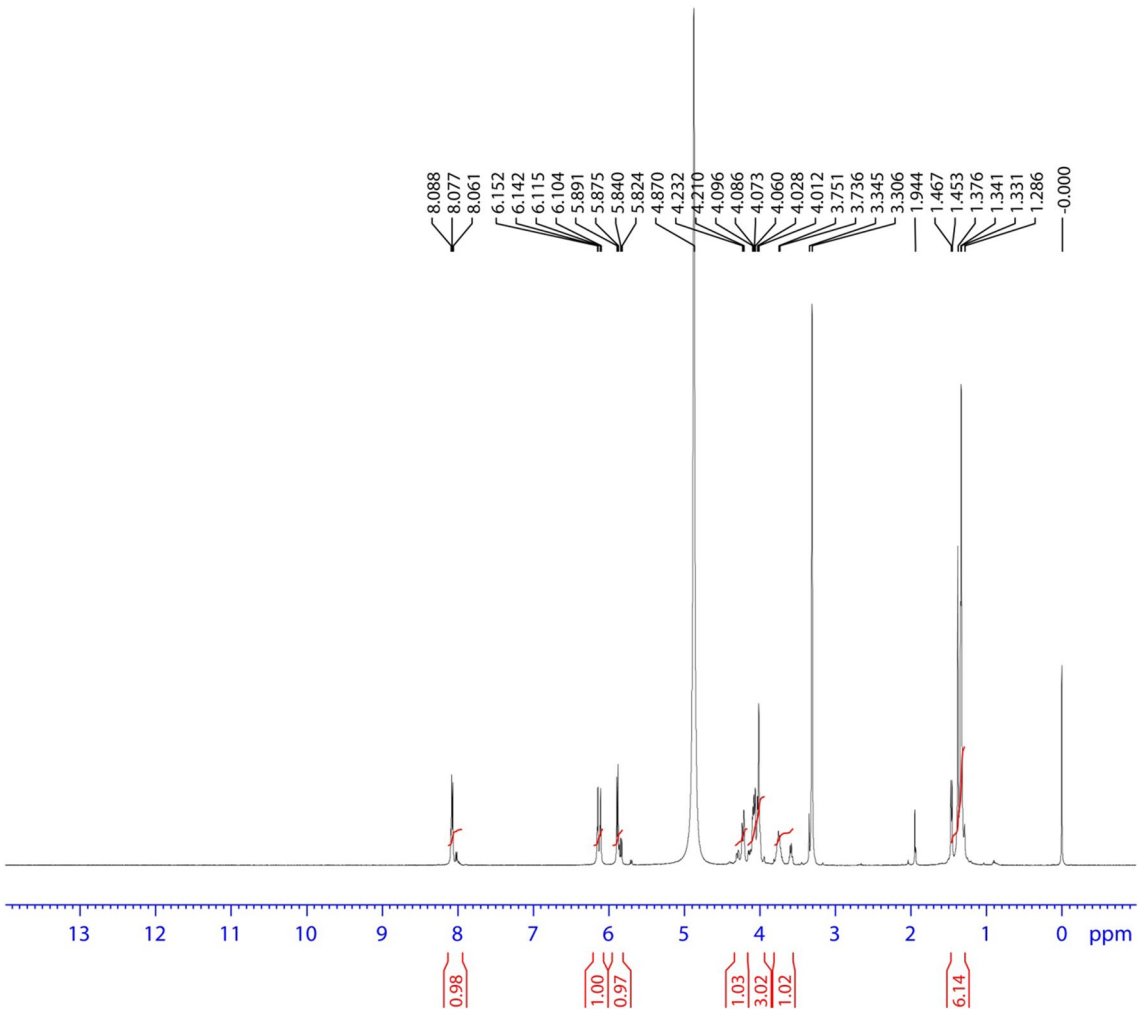


Fig. 13 ^{1}H NMR spectrum of base degradation product-B

An inner-sphere mechanism was considered for the electron transfer of the protonated and deprotonated forms of sofosbuvir via the formation of an intermediate between sofosbuvir anion and the bromium ion (Br^{+}) that produced by heterolytic dissociation of N–Br bond in *N*-bromosuccinime in a step preceding the rate determining steps.

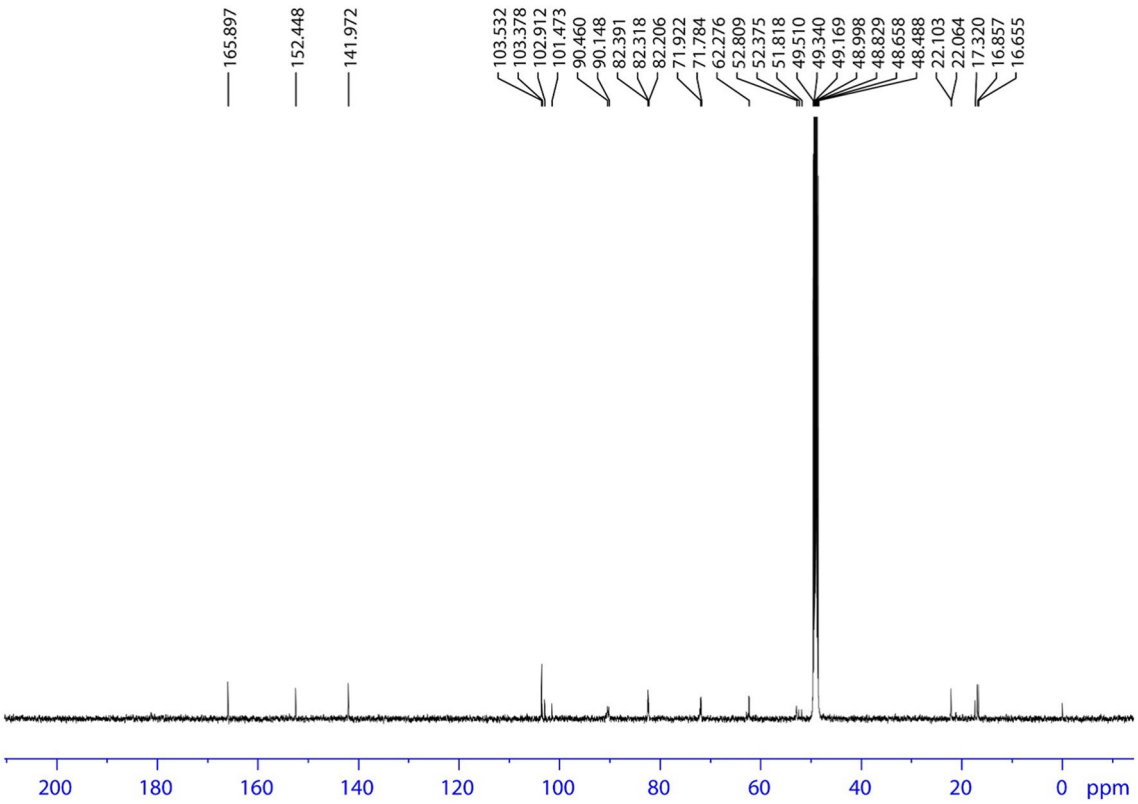


Fig. 14 ^{13}C spectrum of base degradation product-B

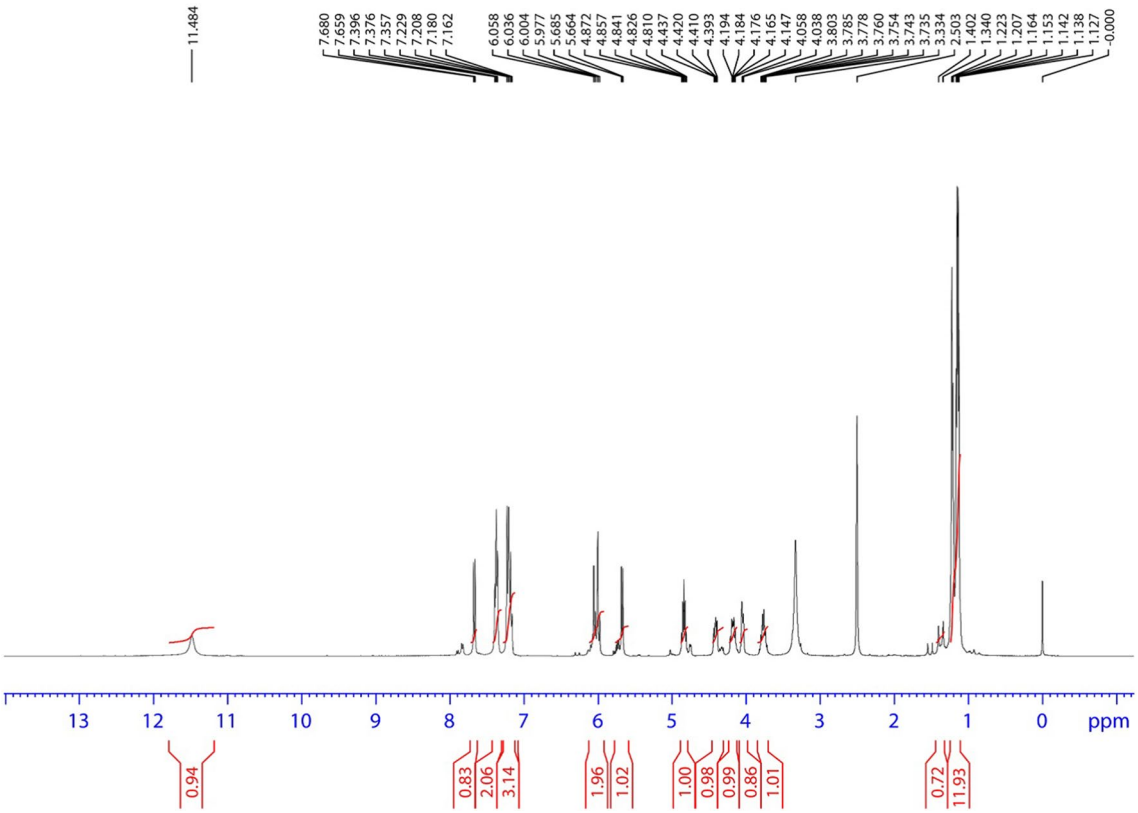


Fig. 15 ^{1}H NMR spectrum of oxidative degradation product

Table 4 Values of a and b at different temperatures

T (°C)	a (mol dm^{-3} s)	b ($mol^{-1}dm^3$)
10	15.19	1963.38
15	18.60	2105.49
20	25.20	2509.82
35	41.39	3287.21

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Compliance with Ethical Standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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