

# Free Radical Scavenging Activity of Five Benzoic Acid Derivatives: A Theoretical M06-2X Study

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## Abstract

The meta hybrid M06-2X functional combined with the 6-311++G(d,p) basis set are used to investigate the antioxidant activity of five benzoic acid derivatives naturally occurring in several plant food: gallic acid, para-hydroxybenzoic acid, protocatechuic acid, syringic acid and vanillic acid. To assess the antioxidant properties of these compounds, three mechanisms of free radicals scavenging are considered, namely the hydrogen atom transfer (HAT), stepwise electron transfer proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET) models. The effect of polar environments on the antioxidant power of these compounds is mimicked in water and methanol using the IEF-PCM solvation method. Our findings suggest that HAT is the preferred mechanistic pathway in gas phase, while SPLET is favoured in polar mediums. Protocatechuic and gallic acids are the most active in gas and polar solutions respectively, whereas PHBA is the least active in all the environments considered. The O-H group in para position of the carboxylic group (O3-H for GA and O2-H for the rest) is confirmed to be the most reactive site in gas phase, while in solution it is either of the O1-H site (for PHBA, PCA, SA and VA) or O4-H groups (for GA). The “HOMO-rule” of free radical scavenging ability does not seem to account properly for the antioxidant properties of this set of chemicals.

**Keywords:** Antioxidant activity, HAT, SET-PT, SPLET, frontier molecular orbital

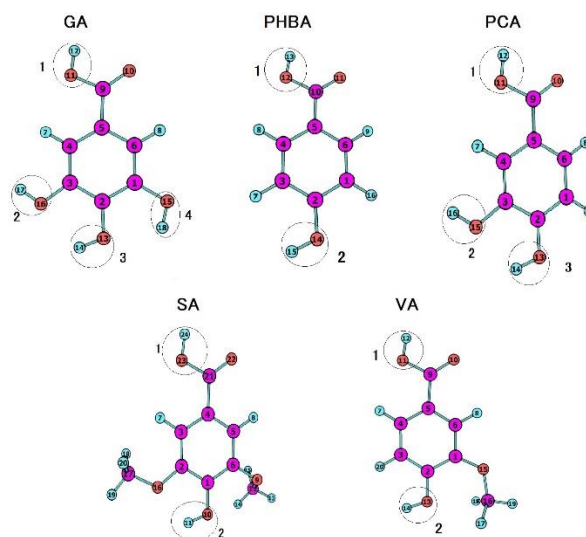
## 1. Introduction

The human body is an excellent autonomous machine where an uncountable number of reactions take place every second to maintain life. Under certain conditions, specific metabolic reactions may produce very reactive entities bearing an unpaired electron and free to move throughout the body [1]. For example, it is known that viral infections tend to stimulate the production of free radicals [2]. When released in small amounts, free radicals are often beneficial to the body as they are involved in oxygen-dependent reactions and aerobic respiration [3]. In this first character, free radicals are friendly products of normal cellular metabolism and play incredible roles in the immune defense system, in several cellular signaling pathways, in mitogenic response as well as in redox regulation [4]. However, due to their strong oxidative character, excessive amounts of free radicals may get out of control and cause severe damages to biological macromolecules such as lipids [5], proteins [6], and DNA [7], which may ultimately lead to apoptotic or necrotic cell death [8].

Dangerous free radicals are often annihilated in the body thanks to an automatic response mechanism relying on the presence of molecules called antioxidants. These molecules exhibit the ability to scavenge free radicals and alleviate the damage provoked by these “hot” chemical entities [9]. Normally, a balance between free radicals and antioxidants is necessary for adequate physiological function. If free radicals become greater than the ability of the body to control them, this gives rise to a situation known as oxidative stress [10], which favors the development or acceleration of many deadly diseases such as Alzheimer's disease [11], cancer [12], and liver fibrosis [13]. In case the human body seems not to be producing enough antioxidants, the recommendation is to supply it with external sources of antioxidants on a regular basis to maintain the equilibrium and tackle the oxidative stress. It must be noted that many antioxidants occur naturally in plants [14][15][16][17][18], animals [19][20], and microorganisms [21][22] or may be synthesized by chemical means [23][24]. Thus, with the perpetual need to identify more effective

antioxidant candidates to integrate into the preventive or therapeutic pipeline of several pathologies, the isolation and synthesis of new antioxidants is a hot research domain attracting the interest of a myriad of scientists [25][26][27][28].

During the past decades, a number of studies have excellently documented the antioxidant activity of benzoic acid derivatives and many more investigations are still popping up [29][30][31][32]. Following the same trend, we report in the present study an *in-silico* investigation of the antioxidant power of five benzoic acid derivatives including gallic acid (GA), para-hydroxybenzoic acid (PHBA), protocatechuic acid (PCA), syringic acid (SA), and vanillic acid (VA) (Figure 1).



**Figure 1.** Schematic representation of the five compounds investigated, as well as the atomic numbering and active site (OH groups) labelling considered in this study

It is worth noting that all of these compounds are present in plant food where they usually occur in bound form as components of complex structures like lignins and hydrolysable tanins, or attached to cell walls and proteins [33][34]. Although these molecules have been the subject of separate previous works [35][36], the present study draws its peculiarity from the fact that it involves all of them in a comparative discussion of their antioxidant activity in gas and polar phases at the M06-2X/6-311++G(d,p) level, and following three different mechanisms of free

radical scavenging. The antioxidant activity of each compound is assessed using thermodynamic parameters defined with respect to each mechanism. Then, the collected data are used to discuss the most preferred pathway in each environment, before inferring the most likely order of free radical scavenging activity. Note that spin densities and frontier molecular orbitals are computed to get insight into the global and local reactivity of the five compounds.

## 2. Methodology

### 2.1. Mechanism of free radical scavenging activity

Three main mechanisms have been advanced to explain the free radicals scavenging action of (poly)phenolic antioxidants. Although the net result of the three mechanisms is the same, i.e. the formation of less harmful radicals, it is possible that under certain conditions one of the possible mechanisms may prevail [17].

In the first mechanistic pathway, known as the hydrogen atom transfer (HAT) and summarized in equation 1, an ArO-H antioxidant donates its H atom to a hot free radical R<sup>\*</sup>, which is converted into a neutral species RH alongside a less reactive ArO<sup>\*</sup> radical [37]-PT. In the framework of the HAT mechanism, the antioxidant activity is readily assessed by computing the Bond Dissociation Enthalpy (BDEs) of the O-H bonds of the antioxidant. Since BDEs measure the amount of energy required to break a bond in a homolytic fashion, small BDEs are to be associated with higher antioxidant activity [15].

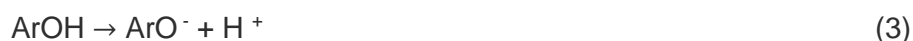


Equation 2 illustrates the second mechanism, i.e. the stepwise electron transfer proton transfer (SET-PT) route. On this path, an ArO-H molecule consecutively withdraws one electron and a proton onto the free radical, leading to the same products as in the HAT route [38]. The ionization potential (IP) and proton dissociation enthalpy (PDE) of the antioxidant are the most

used indicators of the antioxidant activity in this context, low IPs and PDEs being associated with great antioxidant properties [39].



The third mechanistic pathway (equations 3-5) has been named sequential proton loss electron transfer (SPLET) as it involves the heterolytic dissociation of O-H bond before the subsequent shift of a single electron from  $ArO^{\bullet}$  to the free radical [40]. In so far as this mechanism is concerned, the proton affinity (PA) and electron transfer enthalpy (ETE) are the most suitable thermodynamics parameter to discuss the activity of antioxidants. It is widely admitted that the lower the PAs and ETEs are, the higher the antioxidant activity [41].



## 2.2. Computational details

Following each of the three mechanisms of free radical scavenging, we investigate here the antioxidant activity of GA, PHBA, PCA, SA, and VA in vacuum (gas phase) and solution (water and methanol) using the Gaussian 09 software [42]. All the structures, i.e. parent molecules, radicals, cations, and anions, were fully optimized using the meta hybrid M06-2X functional in conjunction with the 6-311++G(d,p) basis set. Note that the M06-2X/6-311++G(d,p) level of theory has been successfully applied in numerous previous studies on antioxidant activities and was found to provide reliable results [35][43]. All the structures returned upon optimization were confirmed by vibrational frequency calculations to be real minima on the potential energy surface. Moreover, Noncovalent Index (NCI) calculations were performed to probe intramolecular noncovalent contacts in the gas phase structure of the antioxidants.

Considering a typical antioxidant noted ArOH, equations 6-10 are used to calculate BDE, IP, PA, PDE, and ETE values. In the gas phase, formation enthalpies of 1.483 kcal/mol and 0.752 kcal/mol were used for the proton and electron respectively as obtained by Bartmess through numerical solution of Fermi-Dirac statistical mechanics equations [29]. Solvation enthalpies of the proton and electron in water and methanol were retrieved from a benchmarking study by Rimarčík and coworkers [44]. Solvent contributions to the total enthalpies were accounted for using the integral equation formalism IEF-PCM method [45][46] as implemented in the Gaussian 09 software with default settings at 298.15 K and 1 atmosphere. Note that the IEF-PCM solvation model is not only computationally affordable, but also known to provide acceptably accurate results [47].

$$\text{BDE} = H(\text{ArO}^\bullet) + H(\text{H}^\bullet) - H(\text{ArOH}) \quad (6)$$

$$\text{IP} = H(\text{ArO}^{+\bullet}) + H(e^-) - H(\text{ArOH}) \quad (7)$$

$$\text{PDE} = H(\text{ArO}^\bullet) + H(\text{H}^+) - H(\text{ArOH}^{+\bullet}) \quad (8)$$

$$\text{PA} = H(\text{ArO}^-) + H(\text{H}^+) - H(\text{ArOH}) \quad (9)$$

$$\text{ETE} = H(\text{ArO}^\bullet) + H(e^-) - H(\text{ArO}^-) \quad (10)$$

To understand the local and global reactivity of the five benzoic acid derivatives, their frontier molecular orbitals were computed at the M06-2X/6-311+G(d,p) level and examined. The visualization was performed using Chemcraft [48] and VMD [49].

### 3. Results and Discussion

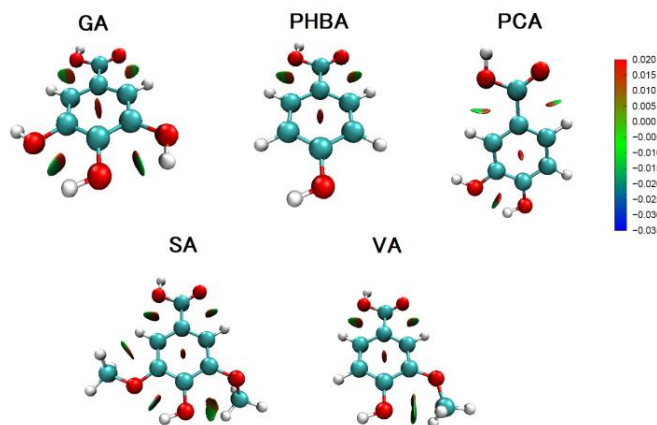
#### 3.1. Optimized geometries

Parent molecules were fully optimized at the M06-2X/6-311++G(d, p) level in the gas, H<sub>2</sub>O and MetOH phases. Figure 1 presents their structures as well as the atomic numbering and convention used herein to differentiate the –OH groups of each molecule. Owing to the distinct electronic surroundings of OH groups as shown in Figure 1, one should expect them to exhibit different reactivity towards free radicals. It is almost universally admitted that the greatest reactivity of benzoic acid derivatives is held in the –OH group in para position of the carboxylic group holds [50]. In the present study, this observation is discussed in the context of all the three mechanisms.

Table S1 collects O-H and C=O bond lengths in all the parent molecules obtained at the M06-2X/6-311++G(d, p) level. It is immediately clear from this table that the carboxylic OH group **1** is slightly longer than the others and measures on average 0.967 in the gas phase and 0.968Å in both water and methanol solutions. Moreover, the length of C=O bonds is estimated to roughly 1.202Å in vacuum, and 1.208Å in both H<sub>2</sub>O and MetOH. These values suggest that both the O-H and C=O are weakly stretched in polar environments, with elongations reaching up to 0.003Å and 0.006Å respectively.

In order to probe intramolecular noncovalent interactions in the neutral structure of the five antioxidants, NCI calculations were carried out in gas phase for all the compounds. The NCI plots obtained at the 0.05 isosurface are given in Figure 2.





**Figure 2.** NCI isosurfaces obtained using the M06-2X/6-11++G(d,p) electron density and reduced density gradient.

Figure 2 not only confirms the presence of intramolecular noncovalent interactions in the structure of all the five molecules, but also reveals that the two O atoms of the carboxylic groups are engaged in non-classical O...H-C hydrogen bonding interactions with the H atoms at ortho position. In line with the common interpretation of NCI plots [51], the greenish color of the elliptical slabs indicate that these intramolecular contacts are weak attractive interactions.

### 3.2. HAT mechanism

In the HAT mechanism of antioxidant activity, an H atom is directly transferred to the free radical by homolytic cleavage of the O-H bond [5]. The bond dissociation enthalpy (BDE) is the most used thermodynamics parameter to appraise the ease with which this breakage occurs. Low BDEs values are then related to high antioxidant power. Table 1 collects calculated BDEs of the five antioxidants in gas, H<sub>2</sub>O, and MeOH phases.

At first glance, this table reveals that BDEs span over a 33.7 kcal/mol range of values going from 78.2 to 111.9 kcal/mol. This range is in good agreement with other theoretical works on other systems [52][53] and gives more credit to the data reported here. In the gas phase, the lowest BDE is associated with the OH group **3** of PCA, while the highest corresponds to the

carboxylic OH group 1 of VA. Based on lowest BDEs values, one can advance the following order of antioxidant activity in the gas phase: PHBA < SA < VA < GA < PCA. In addition, it is worth noting that the OH group in para position exhibits the lowest BDEs in all the compounds and should therefore be the most active in the gas phase through the HAT pathway.

Turning now on the results in solution, Table 1 reveals some interesting insights. Looking first at the raw BDE differences between the gas phase and solution, one should notice that the hydrogen abstraction ability of these compounds is only slightly affected by the two polar solvents. Indeed, BDEs deviations between the gas phase and solution are lower than 6, 11, 6, 7 and 8 kcal/mol for GA, PHBA, PCA, SA and VA respectively. These fluctuations evaluate to less than 10% of the dissociation enthalpy in the gas phase, and agree well with previous findings of the antioxidant activity of benzoic acid derivatives [35]. Moreover, GA appears to be the most active in H<sub>2</sub>O and MeOH solutions, with the lowest BDE of 78.2 and 80.4 kcal/mol respectively in water and methanol attributed to the OH group 3. These values have the same ballpark as the BDEs of GA obtained experimentally by Denisova et al [54], and Alberti et al [55], who found 83.03 and 81.00 kcal/mol in methyl linoleate and acetonitrile/chlorobenzene respectively. Further, the trend of antioxidant activity in solution is slightly different as GA gets more active than PCA [PHBA < SA < VA < PCA < GA].

**Table 1.** Bond dissociation enthalpies (BDE in kcal/mol) of the investigated benzoic acid derivatives in vacuum and solution (water and methanol) were computed at M06-2X/6-311++G(d, p) level.

Antioxidant	Medium	O-H(1)	O-H(2)	O-H(3)	O-H(4)
<b>GA</b>	gas	110.8	80.2	80.3	88.0
	water	105.1	80.7	78.2	83.8
	methanol	107.9	82.8	80.4	86.1
<b>PHBA</b>	gas	110.6	88.1		
	water	110.8	87.8		
	methanol	121.4	89.9		
<b>PCA</b>	gas	110.8	87.4	78.8	
	water	105.1	83.7	79.4	
	methanol	107.8	86.0	81.5	
<b>SA</b>	gas	110.5	83.5		
	water	104.1	84.4		
	methanol	106.8	86.4		
<b>VA</b>	gas	111.9	80.7		
	water	104.6	-		
	methanol	109.2	85.7		

The BDEs values reported in Table 1 enable to identify the most active site and compound in gas and polar media in the context of the HAT mechanism. However, they do not indicate why this should be so. After homolytic breakage of an O-H bond, the single electron left on the O atom delocalizes over the molecule and one can assume that the more it spreads away from its initial position, the higher the stability of the radical and the lower should be the BDE. Therefore, the difference in BDE values may be explained based on the distribution of spin densities [56].

In order to comprehend the origin of the different BDEs of O-H bonds in gas phase, spin density distributions of the radicals were calculated at the M06-2X/6-311++G(d, p) level and are here listed in Table 2. Inspection of this table asserts that for the same compound, the OH group in para position has the lowest spin density estimated to 0.66, 0.67, 0.68, 0.71 and 0.61 respectively for GA, PHBA, PCA, SA and VA, and should lead to the most stable radical. This

observation is consistent with the BDEs listed in Table 1, which showed these OH groups to be the most active. Similarly, the highest spin density of 0.96-0.98 predicted for the carboxylic OH group corroborate well with the high BDEs reported in Table 1.

**Table 2.** Local spin density calculated on the O atom after the homolytic cleavage of O-H bond in the gas, H<sub>2</sub>O and MetOH phases. Values calculated at the M06-2X/6-311++G(d,p) level.

Antioxidant	O(1)	O(2)	O(3)	O(4)
GA	0.97	0.70	0.66	0.68
PHBA	0.97	0.67		
PCA	0.96	0.70	0.68	
SA	0.98	0.71		
VA	0.97	0.61		

However, it must be emphasized that this analysis relying on local spin densities only holds when it comes to identifying the most active site within the same compound, but does not account for the order of lowest BDEs throughout the whole dataset of five compounds. For example, the spin density on the OH group **2** of VA (0.61) is lower than that of the OH group **3** of PCA (0.68), while the BDE of the former is 1.9 kcal/mol greater than that of the latter. This finding is consistent with the results reported by Wang *et al* on a series of 2,3,4-trimethoxy chalcones [57].

### 3.3. SET-PT mechanism

In terms of SET-PT mechanism, ionization potentials (IPs) and proton dissociation enthalpies (PDEs) serve to investigate the antioxidant power. A rule of thumb is that, the lower the IPs and PDEs, the greater the antioxidant activity. Table 3 reports the calculated IPs and PDEs of the five antioxidants in the gas phase and H<sub>2</sub>O and MetOH solutions at the M06-2X/6-311++G(d,p) level.

Results in Table 3 reveal that the IP values of the five antioxidants range between 185.5 and 195.5 kcal/mol in gas phase, and between 121.8 and 132.9 kcal/mol, and 127.1 and 138.3

kcal/mol respectively in water and methanol. These values are in the same range as those obtained in previous studies [58][59]. Noteworthy is that IPs values suggest either of the following orders of antioxidant activity which are far different from the one obtained with BDEs: PHBA < PCA~GA < VA < SA, PHBA < GA~PCA < VA < SA and PHBA < VA < GA~PCA < SA in gas phase, H<sub>2</sub>O and methanol respectively. This apparent discrepancy between IPs and BDEs induced patterns is not surprising. It resorts to the fact BDEs are local quantities depending on the relative positions of substituents, whereas IPs are global parameters affected by the structure of the entire molecule [60].

**Table 3.** Ionization potential (IP) and proton dissociation enthalpy (PDE) computed at the M06-2X/6-311++G(d,p) level. PDE values are provided for each site. Values are given in kcal/mol.

Antioxidant	Medium	IP	PDE			
			O1-H	O2-H	O3-H	O4-H
GA	vacuum	194.4	232.3	201.6	201.8	209.5
	water	124.8	27.9	3.6	1.0	6.5
	methanol	130.1	23.9	-1.2	-3.6	2.0
PHBA	vacuum	204.2	222.3	199.8		
	water	132.9	2.4	2.4		
	methanol	138.3	29.3	-2.3		
PCA	vacuum	195.2	231.5	208.1	199.5	
	water	124.6	28.0	6.7	2.4	
	methanol	129.9	24.0	2.1	-2.3	
SA	vacuum	185.5	240.9	213.9		
	water	121.8	29.7	10.4		
	methanol	127.1	25.8	5.5		
VA	vacuum	189.6	238.2	207.1		
	water	122.5	29.7	7.0		
	methanol	134.1	19.2	-4.2		

It is worth noting that IPs decrease drastically from the gas phase to polar media. For instance, the IP of SA plummets from 185.5 to 124.6 kcal/mol on going from the gas phase to

aqueous solution. This indicates that polar solvents considerably facilitate the electron transfer to the free radicals and thus enhance the antioxidant activity. This result is in agreement with many documented studies on antioxidant properties [61]. Furthermore, IP values follow the order water < methanol < gas, which indicate that the stronger the solvent polarity, the higher the electron-donating ability of the molecule [62].

The proton dissociation enthalpy (PDE) characterizes the second step of the SET-PT mechanism and indicates the thermodynamically preferred OH group for deprotonation from the cation radical. It can be seen from Table 3 that the lowest PDE in all the three environments is associated to the OH group in para position, and agrees well with the results based on BDEs. Moreover, PCA has the lowest PDE in gas phase, while this applies to GA in polar solution. Like IP values, there are substantial decrease of PDEs in solution as compared to the gas phase, which may be ascribed to the high stabilization of the positively charged proton in polar solution. Note that, in gas phase, the lowest PDEs suggest the following order of antioxidant activity SA < VA < GA < PHBA < PCA, which is slightly different from the one obtained in solution, namely SA < VA < PHBA < PCA < GA. The negative PDE values predicted for some OH groups may suggest a plausible mediation of the solvent in the heterolytic breakage of the O-H bonds of the radical cation, pulling and favoring the ejection of the proton with no need for extra energy. Wang *et al* reported similar data in their investigation of the antioxidant activity of genistein and its nitro and amino derivatives [57].

### 3.4. SPLET mechanism

Table 4 presents proton affinities (PAs) and electron transfer enthalpies (ETEs) of GA, PHBA, PCA, SA and VA in the gas phase and polar (H<sub>2</sub>O and MeOH) solutions at the M06-2X/6-311++G(d,p) level. In gas phase, PA values are comprised between 329.2 and 344.0 kcal/mol, the lowest and highest values being associated with the O2-H and O4-H groups of GA. It must be

noted that, in gas phase, the O2 –H bond has the lowest PA and should be the most likely to undergo a heterolytic breakage; in polar solution, this is true for the O1-H bond except for GA in water where O4-H has the lowest PA of 30.5 kcal/mol. Inspection of Table 4 reveals that PAs drop considerably when going from gas phase to polar solution ( where PA values are lower than 39.1 kcal/mol), following the order gas phase << water < methanol. This pattern can be related to the high stability of the radical anion and proton in solution as compared to the gas phase. Considering the lowest PA values in gas phase, one can deduce the following order of antioxidant activity PHBA~SA <VA<PCA<GA in gas phase, which changes to PHBA ~ VA < PCA < SA < GA in polar medium.

The second step of the SPLET mechanism corresponds to the fixation of an electron from the free radicals. The capability to trap that electron is estimated by the electron transfer enthalpy defined in equation 10. ETE values listed in Table 4 are in between 58.0 and 89.4 kcal/mol in gas phase and 91.1 and 136.8 kcal/mol in polar solution. In gas phase, site O2 has the lowest ETE value, except in the case of GA for which it is site O4 instead. As such, one can expect these sites (O2 and O4 for GA) to be enriched in extra electron density by a striking free radical. Moreover, looking at the lowest ETE values in gas phase, VA appears to be the ablest to trap an electron from a free radical, followed respectively by GA, PCA, SA and PHBA.

In contrast with PAs, ETE values increase in solution, even if their rise is less pronounced as compared to the decrease of PA values. This finding can be explained by the high solubility of the radical anion ( $ArO^{\cdot-}$ ) in solution. Note that site O2 has the lowest ETE in both solvents for all compounds except GA, for which site O3 and O4 come up with the lowest ETE values of 91.1 and 93.6 kcal/mol respectively in water and methanol respectively. In line with the lowest ETE values, the electron transfer ability of these compounds is as follows: PHBA < SA < PCA < VA ~ GA in both water and methanol, and is very close to that observed in gas phase.

**Table 4.** Proton affinity (PA) and electron transfer enthalpy (ETE) computed at the M06-2X/6-311++G(d,p) level. ETE values are provided for each site. Values are given in kcal/mol.

Antioxidant	Medium	PA				ETE			
		O1-H	O2-H	O3-H	O4-H	O1-H	O2-H	O3-H	O4-H
GA	vacuum	337.3	329.2	330.3	344.0	89.4	67.0	65.9	59.9
	water	39.1	31.8	33.2	30.5	112.0	95.0	91.1	99.4
	methanol	29.3	31.7	30.4	37.9	124.1	96.7	95.5	93.6
PHBA	vacuum	339.3	332.5			87.2	71.5		
	water	31.3	34.4			125.5	99.4		
	methanol	30.2	32.9			136.8	102.5		
PCA	vacuum	337.5	330.5	337.0		89.2	64.3	66.3	
	water	30.9	33.4	35.9		120.2	92.0	93.8	
	methanol	29.7	31.9	34.6		123.6	95.1	96.9	
SA	vacuum	337.7	332.3			88.7	67.8		
	water	30.6	35.9			119.5	94.5		
	methanol	29.4	34.5			122.9	97.4		
VA	vacuum	338.6	331.8			89.1	58.0		
	water	31.0	36.4			119.7	91.6		
	methanol	30.1	35.2			123.2	94.7		



### 3.5. Thermodynamically preferred mechanism

As stated above, IP and PA values characterize the first step of the SET-PT and SPLET mechanisms, while BDEs describe the unique reaction of the HAT mechanism. Having this in mind, previous studies have attempted to determine the thermodynamically preferred reaction pathway of several families of antioxidants by comparing the relative magnitude of BDEs, IPs and PAs [43][63]. The same approach is here applied.

As far as the present study is concerned, BDEs are substantially smaller than IPs and PAs in gas phase. This suggests that the antioxidant activity of GA, PHBA, PCA, SA and VA can be considered as driven by the HAT mechanism in gas phase and corroborates with Urbaniak et al[64]. Therefore, PCA and PHBA should display respectively the highest and lowest antioxidant activity in gas phase. Furthermore, Tables 1, 3-4 reveal that, in polar solution, PAs are very low as compared to BDEs and IPs. This finding indicates that the SPLET mechanism should be the most favorable in water and methanol. As such, GA and PHBA should be the most and least active in polar medium. Note that, regardless of the environment, PHBA seems to be the least active of the set.

### 3.6. Frontier molecular orbitals

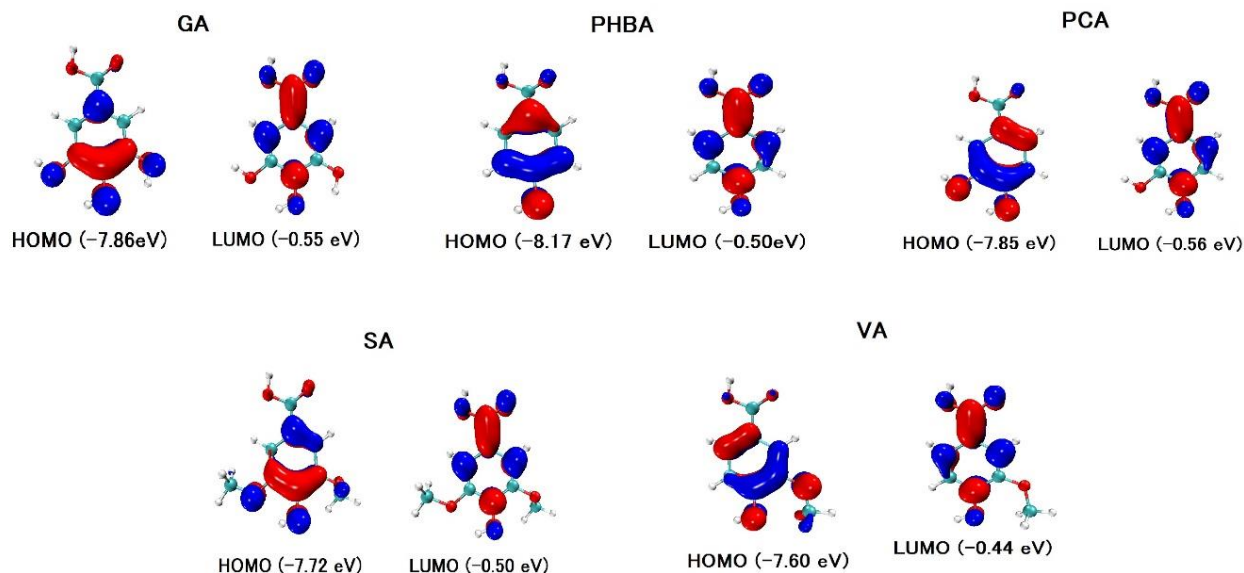
Frontier molecular orbitals (FMOs) are widely used to probe the reactivity of molecular systems [65]. Most often, one can rely on the electronic density distribution in these orbitals to predict the most preferred attack site for free radicals and other reactive agents [63]. More interestingly, previous studies have shown that the free radical scavenging ability of phenolic compounds can be related to the energy of the HOMO such that molecules with higher HOMO energies are likely to be more active due to the stronger electron donating ability [43][63]. To check this “HOMO-rule” and assess the local reactivity of the compounds considered here, FMOs

of the five antioxidants were computed in the three environments. Figure 3 displays the electron density distribution of the FMOs of our antioxidants in gas phase (qualitatively equivalent plots were obtained in solution), as well as the corresponding energies. Electronic energies associated with the HOMO and LUMO in gas phase and H<sub>2</sub>O and MeOH solutions are provided in Table 5.

Figure 3 shows that the electron densities of the HOMO and LUMO are delocalized over a big portion of these molecules. The concentration of this density on O atoms and C atoms of the benzene ring suggests these atoms to be the preferred attack site for free radicals [57]. However, the reactions on C sites has not been considered and may constitute the core of a separate study.

It can be seen from Table 5 that polar solvents stabilize FMOs of the five antioxidant, their effect being more pronounced on the LUMO than the HOMO. Furthermore, note that VA and PHBA are predicted as having the strongest and weakest electron donating abilities in gas phase, with HOMO energies of -7.60 and -8.17 eV respectively, whereas in polar medium, SA has the highest HOMO energy valued at 7.81 eV and should possess the greatest free radical scavenging power as far as the HOMO rule is assumed correct.

However, looking at the HOMO energies collected in Table 5, one may infer the following orders of free scavenging ability: PHBA < GA ~ PCA < SA < VA and PHBA < VA < GA < PCA < SA in gas and polar phases respectively, which do not seem to corroborate with any of the patterns predicted considering either of BDEs, IPs, PDEs, PAs and ETEs. This finding may indicate that the HOMO rule is not absolute, and may be misleading in the discussion of the antioxidant activity. An argument upholding this assumption is that, although the “HOMO-rule” was found to reproduce IPs patterns in the case of quercetin and analogues [43], it does not work for the current benzoic acid derivatives.



**Figure 3.** Frontier molecular orbitals of GA, PHBA, PCA, SA and VA in gas phase at the M06-2X/6-311++G(d,p) level.

**Table 5.** Frontier molecular orbitals (FMOs) energies computed in gas phase and polar (H<sub>2</sub>O and MetOH) solution at the M06-2X/6-311++G(d,p) level. Values are expressed in eV.

Antioxidant	FMO	Gas	Water	Methanol
GA	HOMO	-7.86	-7.89	-7.89
	LUMO	-0.55	-0.67	-0.67
PHBA	HOMO	-8.17	-8.19	-8.19
	LUMO	-0.50	-0.62	-0.61
PCA	HOMO	-7.85	-7.87	-7.87
	LUMO	-0.56	-0.66	-0.65
SA	HOMO	-7.72	-7.81	-7.81
	LUMO	-0.50	-0.69	-0.68
VA	HOMO	-7.60	-8.11	-8.10
	LUMO	-0.44	-0.68	-0.67

#### 4. Concluding remarks

The antioxidant activity of five benzoic acid derivatives has been investigated at the M06-2X/6-311++G(d,p) level, following three possible mechanisms of antioxidant activity in gas and polar phases. Our findings suggest that the hydrogen atom transfer (HAT) is the preferred mechanistic pathway in gas phase, while the sequential proton loss electron transfer (SPLET) is favored in polar mediums. Protocatechuic and gallic acids (PCA and GA) are the most active in gas and polar solutions respectively, whereas PHBA is the least active in all the environments considered. Solvents are found to induce considerable changes in the enthalpies of charged species, explaining some drastic variations in proton affinities (PA) and Proton Dissociation Enthalpies (PDE) when going from gas phase to polar solutions. The O-H group in para position of the carboxylic group (O3-H for GA and O2-H for the rest) is the most reactive site in gas phase, while in solution it is either the O1-H site for PHBA, PCA, SA and VA, or O4-H for GA.

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