BRCORP
TECHNOLOGY | INNOVATION | INDUSTRY

5th World Conference on
Applied Sciences, Engineering & Technology
02-04 June 2016, HCMUT, Vietnam

Transformation Products of Aqueous Sulfamethoxazole by ^{60}Co Gamma Irradiation - A Combined Computational and Experimental Study

NAM D. LE[1,2], TRANG T.T. LE[3], TAM V.T. MAI[4], LAM K. HUYNH[5], THANG M. NGO[1]
[1]*Department of Physico-chemical Engineering, HCM University of Technology, Ho-Chi-Minh City, Vietnam*
[2]*Center for Analyzing, Classifying Imported & Exported Goods – Ho-Chi-Minh City, Vietnam*
[3]*Key Lab of Chemical Technology, Vietnam National University, Ho-Chi-Minh City, Vietnam*
[4]*Institute for Computational Science and Technology, Ho-Chi-Minh City, Vietnam*
[5]*Department of Biotechnology, International University, Ho-Chi-Minh City, Vietnam*
Email: 7140193@hcmut.edu.vn, hklam@hcmiu.edu.vn, nmthang@hcmut.edu.vn

Abstract: Radiolysis of sulfamethoxazole (SMX) was investigated in aqueous samples with different initial conditions: $[SMX]_0$ (20 µM ÷ 140 µM), pH_0 (2.0 ÷ 11.0), $[H_2O_2]_0$ (0 ÷ 10 mM) and absorbed doses (D, 0.3 kGy ÷ 5.0 kGy). Five transformation products (TPs) of SMX were experimentally identified and evaluated using HPLC/UV and LC-QTOF-MS, respectively. Compared to the precursor SMX, they possess higher polarities as well as molar masses (m/z = 270- 304) with –OH group preferentially attached to the benzene ring. The calculated hydrophobicity abilities of SMX and its products showed a linear correlation with the corresponding chromatographic retention times. Under the considered experimental conditions, no significant effects of the initial sample compositions on the TPs' identities were observed. It was found that the abundances of all 5 TPs increased with the increase of the absorbed doses up to about 1.0 kGy. Higher absorbed doses resulted in the decrease of their abundances, reaching the non-detectable levels at D = 5.0 kGy. Based on the combined experimental and computational results, possible transformation schemes of SMX are briefly proposed and compared to the literature.

Keywords: *Sulfamethoxazole, radiolysis, transformation products, transformation schemes, hydrophobicity.*

Introduction:

Antibiotic residues in surface- and groundwater has been a serious environmental issue [1-3]. As most antibiotics for human healthcare in Vietnam are still available without prescriptions, higher levels of antibiotic residues, e.g., sulfamethoxazole (SMX), were observed [4, 5]. SMX, whose structure is shown in Fig.1, belongs to the frequent targets of antibiotic treating efforts [6-12]. In general, SMX is considered persistent in natural waters as well as in conventional wastewater treatment procedures. For effective removal of SMX residues from water, advanced oxidation processes (AOPs) should be applied. The characteristic feature of AOPs is the *in-situ* generation of hydroxyl radicals ●OH, the most oxidative agent. On the other hand, the ●OH groups react non-selectively with the sample components, resulting in a variety of intermediates and end-products through several reaction pathways. In many cases, values of total organic carbon (TOC) in the treated samples decreased substantially slower than that of the main target pollutant (i.e., SMX), indicating that the intermediates and/or end-products were even more persistent than their precursors is in question. From the viewpoint of water treatment/reuse, the organic pollutants should be mineralised. As the total mineralization of organic pollutants by AOPs is rarely achieved, details about the transformation pathways and their products (TPs) should be investigated and identified. Recently, the identities of TPs have been examined using various types of sophisticated mass spectrometers (MS) coupled to liquid chromatographs (LC). However, the published TPs might be different or even controversy, depending on the actual MS configuration in use and the analysts' skills [13, 14]. Besides, no comprehensive discussion had been devoted to retention times of the identified TPs, although they indicate an order of TPs' polarities.

The LC performed on analytical columns packed with a commercially available solid phase containing long hydrocarbon chains (C^{18}) chemically bound onto silica. The C^{18} hydrocarbon provides a hydrophobic micro-environment and compounds moving through this system, partition between the stationary (non-polar) and mobile (polar) phases according to their physicochemical properties. The chemicals are retained in proportion to their hydrocarbon – water partition coefficient, with water-soluble chemicals eluted first and oil-soluble chemicals last.

Gamma irradiation is a new alternative to the conventional AOPs in wastewater treatment as it also generates ●OH in-situ. It is more advantageous compared to the conventional AOPs as virtually no further chemicals are necessary. Recently, two research groups have reported that quantitative removals of SMX from water were achieved after irradiation with doses of 1.0 kGy [11] or 5.0 kGy [12], respectively. However, according to Wang *et al.* [11] the TPs possess hydroxyl groups preferentially attached to isoxasole ring, while Sági *et al.* [12] concluded attaching to benzene ring is preferred.

Recently, computational science has become a powerful tool that helps to explain and predict experimental results; therefore, this paper presents a combined approach, experimentation and

computation, to study the radiolysis of SMX. In particular, the experiments on radiolysis of SMX in water were carried out using HPLC/DAD and LC-TOF-MS, which is more suitable for identifying unknown TPs than MS detectors used in previous works [11-14]. Computationally, the molecule structures of SMX and its identified TPs were optimized using Gaussian09 [16], and consequently served to evaluate their hydrocarbon – water partition coefficients by means of ACD Labs method [17]. The calculated values log*P* were discussed with the corresponding experimental chromatographic retention times.

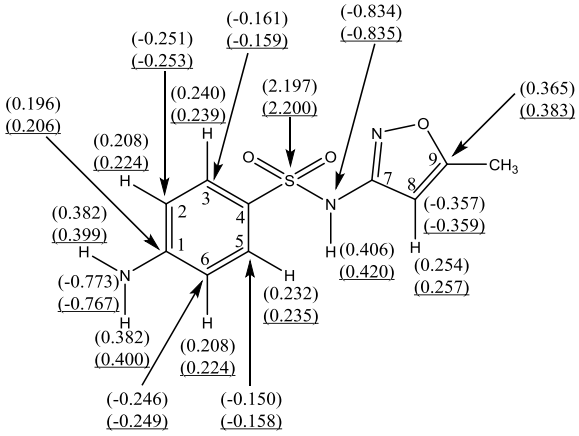

Figure 1: Sulfamethoxazole structure and its charge population for some presented atoms using natural bond orbital (NBO) [18, 19] approach, were obtained at B3LYP/6-311++G(d,p) [20, 21] level of theory in gas-phase.

(The underlined values were calculated in water solvent using the polarizable continuum model (PCM) [22]).

Materials and Methods:

All chemicals were of analytical grade, purchased from Merck and used without further purification: Sulfamethoxazole (99%), sulphuric acid (98%), nitric acid (65%), sodium hydroxide (>99%), hydrogen peroxide (30%). Formic acid and acetonitrile of LC/MS grade, and MQ water were used to prepare the mobile phase for LC analysis. Double distilled water was used to prepare the sample solutions.

Irradiation was performed in Center of Irradiation Technology – Nuclear Research Center Dalat, using 10 ml samples in tightly closed 12 mL - glass tubes and Gamma Chamber–5000 (BRIT, India) ^{60}Co with activity of 3000 Ci and dose rate of 2.8 kGy/h. Solutions were irradiated with 6 doses (0,3 kGy ÷ 5,0 kGy) and 3 replicates for statistic evaluation. All samples before and after irradiation were analyzed with HPLC/DAD. Several selected samples were analyzed with LC/ESI-TOF-MS.

HPLC/DAD analyses were performed using Agilent 1290 infinity series with reversed phase column Agilent Poroshell 120 EC–C18, (4,6 mm x 100 mm; 2,7 µm) and guard column Agilent Eclipse Plus C18 (2,1mm x 5 mm; 1,8µm); the mobile phase was constant of H_2O/ACN = 85/15 (v/v), flow-rate 1.0 ml/min, sample volume 20 µL. The wavelength of DAD was set at 270 nm.

LC/MS analyses were performed using Agilent 1290 infinity series LC-ESI-(Q)TOF–MS/MS G6530A with reversed phase column Agilent poroshell 120 C18 (4,6 mm x 250 mm; 2,7 µm) and guard column Agilent poroshell 120 EC – C18 (4,6 mm x 5 mm; 2,7 µm). The mobile phase was constant of 0,1% HCOOH/ACN = 70 : 30 (v/v), flow-rate 0.25 ml/min, sample volume 20 µL. Detector MS G6530A was set at positive mode, capillary voltage 3500 V, fragmentor voltage 200 V; drying gas temperature and flow-rate 365^{0}C and 12 L/min, respectively; nebulizer pressure 60 PSI, skimmer voltage 60V, scanning rate 2 spectra /s, duration 500 ms/spectrum, m/z range from 85 → 400.

The structure optimization of SMX and its identified TPs was performed using Gaussian09 [16]. Based on the structures obtained, their hydrocarbon-water partition coefficients, *P*, were calculated using the ACD Labs program by additive-constitutive method [17]. For the retention time, according to Kaliszan *et al.* [15], the prediction of retention parameter from structure of analyte offers the following linear model:

$$t_R = k_1 + k_2' \log P \ , \qquad \text{(eq.1)}$$

where t_R is retention parameter and k_1 and k_2 are regression coefficients. Consequently, a linear relationship between retention time and partition coefficient in forms of t_R vs. $\log P$ can be established.

Results and Discussion:

The HPLC/DAD and LC/TOF-MS methods were optimized after systematic investigations and provided good reproducible results, both for retention times and peak areas. A linear calibration line in the form of SMX peak areas vs. -concentration in 0.1÷100 µM range was obtained with correlation coefficient R of almost identity (cf. Fig. 2).

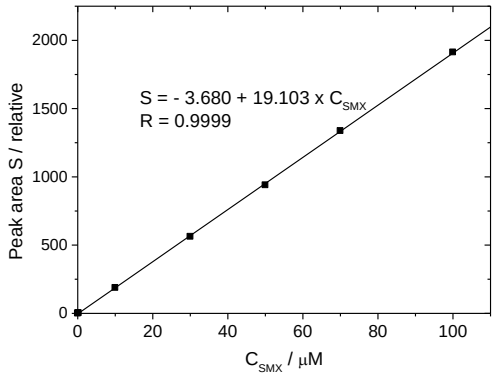

Figure 2: Calibration line for SMX quantification

Comparing to the literature values [11], the selected mobile phase composition of H_2O/ACN = 85/15 (v/v) was slightly higher to get suitable retention times for SMX and peak resolution for its TPs. In general, higher ratios H_2O/ACN in the mobile phase could negatively affect the method limit of quantification. Fig. 3 demonstrates the chromatogram of 0.1-µM SMX samples were still good reproducible. i.e. with this HPLC/DAD procedure, SMX removal yields up to 95% from 20-µM or 99.9% from 100-µM samples could be reliably quantified.

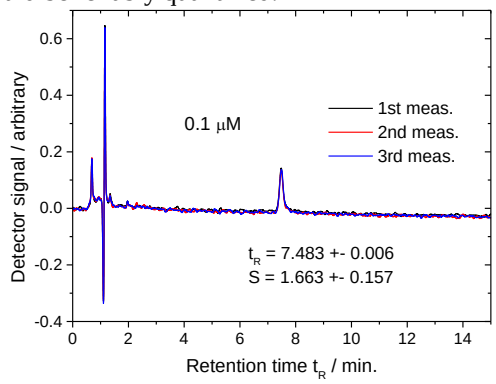

Figure 3: Chromatogram of 0.1-µM SMX sample

As shown in Fig.4, this method was able to trace up to 5 TP-peaks after different absorbed doses. Compare to SMX, all of them have shorter retention times, indicating less polar compounds. This finding was strengthened by the LC/TOF-MS results in Fig.5, as the elution order in both systems follows the same principle.

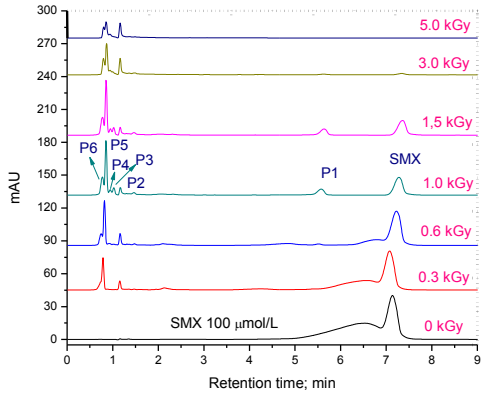

Figure 4: HPLC/DAD chromatogram of 100-µM SMX sample with different doses

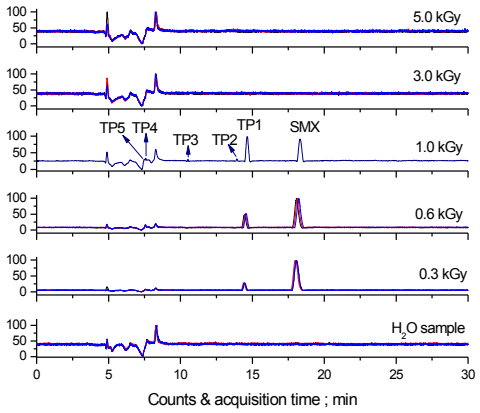

Figure 5: LC/TOF-MS chromatogram of 100-µM SMX sample with 1.0-kGy doses

The results obtained by both methods further confirmed in 100-µM SMX samples the 5 identified TPs accumulated with doses up to 1.0 kGy. Further increasing doses resulted in lowering the TPs signals, which disappeared at 3.0 ÷ 5.0 kGy doses (compare with the signal for pure water sample). Moreover, the peak resolution in Fig.5 is much better than in Fig.4. The reproducibility of retention times evaluated from 3 replicates is also reasonable, (cf. Table 1).

Table 1: Retention times of SMX and its TPs

Compound	Retention time, min.
SMX	18.095 ± 0.111
TP1	14.494 ± 0.059
TP2	13.529 ± 0.392
TP3	10.476 ± 0.013
TP4	7.588 ± 0.022
TP5	7.420 ± 0.007

Table 2 lists the evaluated results from ESI-TOF-MS analysis. All five SMX TPs were identified with very high scores (> 99%) and low mass error (< 2 ppm), so the listed ion forms are strongly confirmed. Based on these results, the SMX transformation pathways were briefly proposed (cf. Fig. 6).

Table 2: TOF-MS results of SMX and its TPs

Compound	Formular	DBE	Target m/z ; Da	Mass error (ppm)	score
SMX	$C_{10}H_{12}N_3O_3S$	6.5	254.0598	3.12	97.53
TP1	$C_{10}H_{12}N_3O_4S$	6.5	270.0548	-1.85	99.05
TP2	$C_{10}H_{11}N_2O_5S$	6.5	271.0379	1.55	99.33
TP3	$C_{10}H_{12}N_3O_5S$	6.5	286.0490	0.76	99.83
TP4	$C_{10}H_{14}N_3O_5S$	5.5	288.0652	-1.16	99.6
TP5	$C_{10}H_{14}N_3O_6S$	5.5	304.0599	-0.39	99.95

benzene ring, resulting however only one peak. i.e.,

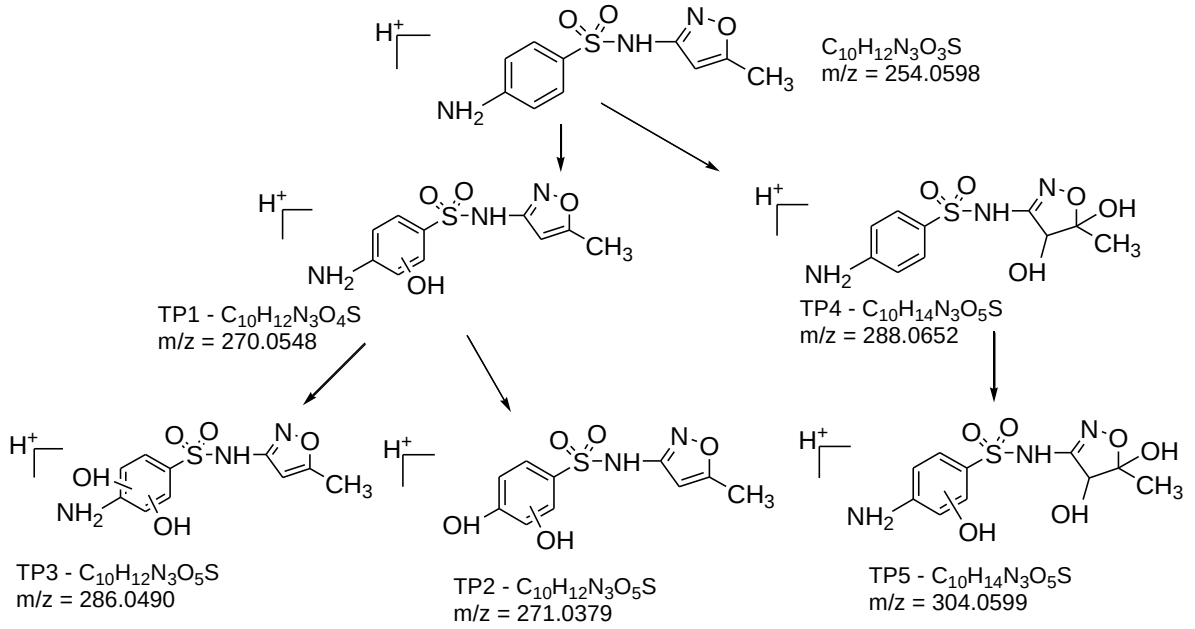

Figure 6: Transformation pathways of SMX in gamma irradiated aqueous samples

Wang *et al.* [11] reported six TPs in gamma irradiated SMX samples. However, two of them were fragments from splitting the isoxazole ring from SMS molecule. Both these two SMX fragments, as well as the third suggested TP with the isoxazole ring being open/broken were not registered in our work. The corresponding mono-hydroxylated substituents also differ from our TP1 and TP2 by attaching –OH group to the isoxazole ring. The last one is consistent with our TP4, but the denoted m/z was 286.1 as our TP3, which has two –OH groups attached to the benzene ring. These discrepancies could have 2 reasons: (1) Wang's gamma source probably had higher dose rate than ours so the ●OH density was higher, which caused more intensive SMX transformation including molecule splitting and/or the isoxazole ring open/broken; (2) Wang's MS configuration caused more intensive fragmentations of the SMX TPs than our TOF-MS. In general for identifying unknown compounds the TOF-MS is considered more suitable than another MS configurations [13, 14].

In accordance with reason (1) mentioned above Sági *et al.* [12] reported the isoxazole ring fragment as one of main SMX TPs by gamma irradiation with a higher dose rate, 6.2 kGy/h. However, the benzene ring fragment did not appear. It is questionable because the benzene ring is generally considered more stable than the isoxazole. Further, two mono-hydroxylated main SMX TPs were reported, with an –OH group attached either to the benzene ring as our TP1, or attached to the isoxasole ring which actually should be the precursor of our TP4. In addition, there were two double hydroxylated SMX TPs, both attached to the benzene ring resulting m/z = 286, but with 2 different retention times. Our TP3 also has the same m/z and both – OH groups attached to the benzene ring, resulting however only one peak. i.e., the abundances of TP3's isomer and TP4's mono-hydroxylated precursor in our samples were too low. Further investigation should be carried out to trace these TPs and elucidate the transformation pathways.

In this work, attention is first time focused to elucidate the consistency between the structures of identified compounds and their chromatographic retention times. The hydrocarbon – water partition coefficients Ps could be estimated from chemical structures of related compounds according to some logarithms [23, 24]. Among them, the most widely accepted method is classified as the "additive method", where a molecule is dissected into basic fragments and its logP value is obtained by summing the contributions of each fragment. Using the ACD Labs program including additive-constitutive method [17], a fairly good linear correlation between the calculated logPs, and the experimental retention times was obtained in Fig. 7 by Kaliszan's model [15], which additionally confirms our TPs' identification.

Table 3: Calculated logPs of SXM and its selected TPs[a]

Compound	LogP
SMX	0.89 ± 0.42
TP1a	0.15 ± 0.43
TP2a	0.80 ± 0.44
TP3a	-0.57 ± 0.45
TP4	-1.26 ± 0.70
TP5a	-2.00 ± 0.70

[a] Selected TPs are structures of TP1a, TP2a, TP3a and TP5a having –OH group(s) attached to benzene ring with *ortho*-position comparing to $–NH_2$ group.

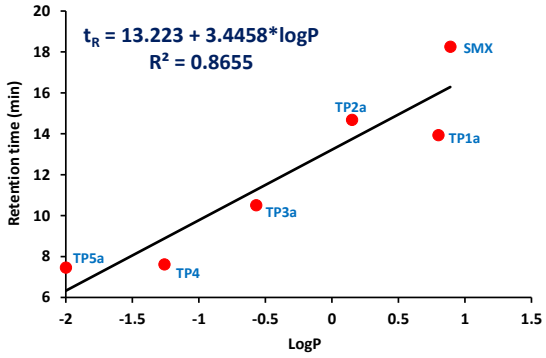

Figure 7: Relationship between the experimental retention time and calculated hydrocarbon-water partition coefficient (LogP).

Conclusions:

Five transformation products of SMX in gamma irradiated aqueous samples were identified using LC/TOF-MS which is more suitable than MS detectors used in other studies. Among the 5 identified TPs the triple hydroxylated TP of SMX (m/z = 304) was discovered for the first time. On the contrary, no fragments from splitting the SMX molecules were registered, showing some of the previously published TPs [11, 12] might be artifacts. Also for the first time, the hydrocarbon-water partition coefficients of identified SMX TPs were computed, showing good linear correlation with their chromatographic retention times, therefore strengthening the TPs' identities. Further calculations to elucidate the SMX transformation pathways are in progress.

Acknowledgements:

Computing resources provided by the Institute for Computational Science and Technology – Ho ChiMinh City and International University, VNU-HCM are gratefully acknowledged.

References:

[1] Kummerer, K., (2009) "Antibiotics in the aquatic environment - a review - part I" *Chemosphere* 75(4), 417-434.

[2] Kummerer, K., (2009) "Antibiotics in the aquatic environment - a review - part II" *Chemosphere* 75(4), 435-441.

[3] Hughes, S. R.; Kay, P.; Brown, L. E., (2013) "Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems" *Environ. Sci. Technol.* 47(2), 661-677.

[4] Hoa, P. T.; Managaki, S.; Nakada, N.; Takada, H.; Shimizu, A.; Anh, D. H.; Viet, P. H.; Suzuki, S., (2011) "Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam" *Sci. Total. Environ.* 409(15), 2894-2901.

[5] Shimizu, A.; Takada, H.; Koike, T.; Takeshita, A.; Saha, M.; Rinawati; Nakada, N.; Murata, A.; Suzuki, T.; Suzuki, S.; Chiem, N. H.; Tuyen, B. C.; Viet, P. H.; Siringan, M. A.; Kwan, C.; Zakaria, M. P.; Reungsang, A., (2013) "Ubiquitous occurrence of sulfonamides in tropical Asian waters" *Sci. Total. Environ.* 452-453, 108-115.

[6] Beltran, F. J.; Aguinaco, A.; Garcia-Araya, J. F.; Oropesa, A., (2008) "Ozone and photocatalytic processes to remove the antibiotic sulfamethoxazole from water" *Water. Res.* 42(14), 3799-3808.

[7] Kim, I.; Yamashita, N.; Tanaka, H., (2009) "Photodegradation of pharmaceuticals and personal care products during UV and UV/H_2O_2 treatments" *Chemosphere* 77(4), 518-525.

[8] Trovo, A. G.; Nogueira, R. F.; Aguera, A.; Fernandez-Alba, A. R.; Sirtori, C.; Malato, S., (2009) "Degradation of sulfamethoxazole in water by solar photo-Fenton. Chemical and toxicological evaluation" *Water. Res.* 43(16), 3922-3931.

[9] Baeza, C.; Knappe, D. R., (2011) "Transformation kinetics of biochemically active compounds in low-pressure UV photolysis and UV/H_2O_2 advanced oxidation processes" *Water. Res.* 45(15), 4531-4543.

[10] Lekkerkerker-Teunissen, K.; Benotti, M. J.; Snyder, S. A.; van Dijk, H. C., (2012) "Transformation of atrazine, carbamazepine, diclofenac and sulfamethoxazole by low and medium pressure UV and UV/H_2O_2 treatment" *Sep. Purif. Technol.* 96, 33-43.

[11] Wang, J. Q.; Zheng, B. G.; Zhang, J. B.; Zheng, Z., (2013) "Degradation of the Antibiotic Sulfamethoxazole in Aqueous Solutions by γ-Irradiation" *Asian J. Chem.* 25(3).

[12] Sági, G.; Csay, T.; Pátzay, G.; Csonka, E.; Wojnárovits, L.; Takács, E., (2014) "Oxidative and reductive degradation of sulfamethoxazole in aqueous solutions: decomposition efficiency and toxicity assessment" *J. Radioanal. Nucl. Chem.* 301(2), 475-482.

[13] Kosjek, T.; Heath, E., (2008) "Applications of mass spectrometry to identifying pharmaceutical transformation products in water treatment" *TrAC-Trends Anal. Chem.* 27(10), 807-820.

[14] Fatta-Kassinos, D.; Vasquez, M. I.; Kummerer, K., (2011) "Transformation products of pharmaceuticals in surface waters and wastewater formed during photolysis and advanced oxidation processes - degradation, elucidation of byproducts and assessment of their biological potency" *Chemosphere* 85(5), 693-709.

[15] Bączek, T.; Kaliszan, R., (2003) "Predictive approaches to gradient retention based on analyte structural descriptors from calculation chemistry" *J. Chromatogr. A* 987(1-2), 29-37.

[16] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; J. A. Montgomery, J.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision A.1*, Gaussian, Inc.: Wallingford CT, 2009.

[17] Spessard, G. O., (1998) "ACD Labs/LogP dB 3.5 and ChemSketch 3.5" *J. Chem. Inf. Comput. Sci.* 38(6), 1250-1253.

[18] Reed, A. E.; Curtiss, L. A.; Weinhold, F., (1988) "Intermolecular interactions from a natural bond orbital, donor-acceptor viewpoint" *Chem. Rev.* 88(6), 899-926.

[19] Weinhold, F.; Landis, C. R., *Valency and Bonding - A Natural Bond Orbital Donor-Acceptor Perspective* Cambridge University Press: Cambridge, U.K.: 2005.

[20] Becke, A. D., (1988) "Density-functional exchange-energy approximation with correct asymptotic behavior" *Phys. Rev. A* 38(6), 3098-3100.

[21] Lee, C.; Yang, W.; Parr, R. G., (1988) "Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density" *Phys. Rev. B* 37(2), 785-789.

[22] Tomasi, J.; Mennucci, B.; Cammi, R., (2005) "Quantum mechanical continuum solvation models" *Chem. Rev.* 105(8), 2999-3093.

[23] Leo, A.; Hansch, C.; Elkins, D., (1971) "Partition coefficients and their uses" *Chem. Rev.* 71(6), 525-616.

[24] Leo, A. J., (1993) "Calculating log Poct from structures" *Chem. Rev.* 93(4), 1281-1306.