ANALYSIS AND TREATMENT OF TRIMETHOPRIM RESIDUES IN WATER BY γ-IRRADIATION

Nam D. Le, Thang M. Ngo

Ho Chi Minh City University of Technology; namleduyhc06@gmail.com, nmthang@hcmut.edu.vn

Abstract - Trimethoprim (TMP) is widely applied in veterinary and also frequently prescribed together with sulfa-methoxazole (SMX) for human medicine. Therefore TMP residues accumulated in agricultural as well as municipal waste water further contaminate surface water. In this paper, the capability of HPLC/UV to detect and quantify TMP residues in water is thoroughly investigated, yielding LOD = 0.06 µM, LOQ = 0.2 µM and very good reproducible calibration line in the concentration range of 2 µM ÷ 100 µM. The resulting procedure is applied to evaluate the capability to treat TMP residues in water (init. 20 µM ÷ 140 µM) by gamma irradiation. Removal yields greater than 99 % are obtained using absorbed doses 0.3 ÷ 3.0 kGy, respectively. Based on the HPLC/UV chromatograms obtained, some aspects of the TMP radio-lytic products in the investigated samples are briefly discussed.

Key words - analysis; antibiotic residues; gamma-irradiation; trimethoprim; water treatment and reuse.

1. Introduction

Residues of pharmaceutical products, especially those of antibiotics in natural aquifers have been detected worldwide [1-4]. A joint research project showed that residues of sulfamethoxazole (SMX) in surface waters in Vietnam are higher compared with those in other countries [5]. As SMX is frequently applied together with trimethoprim (TMP), residues of the latter one in Vietnam's surface water are supposed at elevated levels, too.

Trimethoprim (TMP) with molecule formula $C_{14}H_{18}N_4O_3$ and structure shown in Figure 1 is an antibiotic against a broad spectrum of bacterial species and applied both in veterinary as well as in human medicine (mostly in combination with SMX).

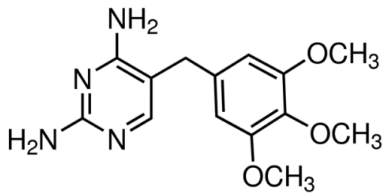

Figure 1. *Molecular structure of TMP*

At ambient conditions (1 atm, 20°C), TMP's solubility in water is about 400 mg/L, which increases with temperature and/or concentration of other organic solvents in the order ethyl acetate, 2-propanol, acetonitrile, ethanol [6]. Moreover, TMP is known to be persistent in conventional wastewater treatment facilities. Therefore, TMP could be involved, accumulated and transported in the environment alongside the water streams.

Attention of several research groups has been focused to treatment of TMP residues in water by diverse methods. E.g. Electro-catalytic degradation on surfaces of carbon electrodes doped by porphyrin manganese was investigated and theoretically validated by means of computational chemistry [7]. Sorption of TMP onto some agricultural soil samples and its desorption by $CaCl_2$ solutions or outflow from an wastewater treatment facility was reported, demonstrating that the local soil- and aquifer compositions play an important role in transport of TMP [8]. Recently, peroxydisulphate initiated by heating to temperatures 50 ÷ 65°C has been applied to activate TMP removal, depending on the sample matrices – while natural organic matters and bicarbonate ions suppress this process, chloride ions accelerate it [9].

By means of photolysis and photo-catalysis, TMP in both distilled water and sea water matrices is relatively stable under natural light illumination. Although an intermediate photolytic product is photosensitive and acted as auto-catalyst, the sample DOC decreases very slowly. Using TiO_2 increases the mineralization degrees of TMP in both matrices, but the rates in sea water are substantially lower because the inorganic components act as hydroxyl radical scavengers [10]. Under similar illuminating conditions by UV-A, UV-C and VUV, hydroxyl radicals play an important role in the samples investigated, enabling up to ~ 73% the total removal yields, while direct photolysis accounts for about ~ 27% [11]. A somewhat more complicated situation is TMP and SMX treatment in urine matrices due to diverse effects of the matrix components [12, 13].

Gamma irradiation using ^{60}Co sources is classified among the advanced oxidation methods as it produces hydroxyl radicals, too. It is applied mainly to discuss the mechanism and intermediate products of TMP (init. 1 mM) transformation by hydroxyl radicals [14]. In a more recent paper, the TMP (init. 20 mg/l ~ 69 µM) removal is reported but focuses on the effects of persulfate concentration 0.5 ÷ 2.0 mM and matrix pH 6,5 ÷ 8,5 [15].

The effect of initial concentration of TMP is not reported in both these 2 publications. Moreover, the HPLC/UV procedures seem very complicated and differ from each other, causing confusion about the reported TMP removal yields. Our paper first focuses on the HPLC/UV procedure for TMP analysis and then on the TMP removal yields depending on its initial concentration and the applied doses.

2. Materials and methods

TMP 99.0% purchased from Sigma-Adrich, formic acid p.a. from Merck, Acetonitrile HPLC grade from J. Baker and other chemicals of analytical grade are used without further purification. Bi-distilled water is used for preparing solutions.

A 1000 µM TMP stock solution is prepared by dissolving 0.0726 g TMP in 250 ml bi-distilled water, stored in dark at ~ 4°C and diluted accordingly to actual samples (TMP conc. in µM: 140, 100, 70, 50, 30, 20, 10, 5, 2, 1, 0.5, and 0.2 µM, respectively) before use.

TMP concentrations are analysed using an HPLC equipment typed Agilent 1290 infinity series, equipped with an Agilent Eclipse Plus C18 guard column (1.8 µm x 2.1 mm x 50 mm), an Agilent Poroshell 120 EC–C18 analytical column (2.7 µm x 4.6 mm x 100 mm) and a diode array detector (DAD). The column was let at room temperature, the injection volume fixed at 10 µl, the wavelength set at 254, 265, 270 and 275 nm. First, various compositions of the mobile phase are tested. Then the mobile phase flowrate, and finally the calibration line is constructed as function of peak volume vs. sample concentration.

Gamma irradiation experiments are conducted at Da Lat Nuclear Research Institute as described in previous paper [16], using a Gamma chamber 5000 (India) ^{60}Co source with dose rate ~ 46.6 Gy/min. Briefly, 8 ml sample (TMP init. conc. 20 µM ÷ 140 µM) is filled in 12 ml glass tube (Hach, USA), tightly closed and irradiated to the pre-determined absorbed dose (0.3 kGy ÷ 3.0 kGy). TMP concentrations before and after irradiation were analyzed using the established procedure and constructed calibration line. Each experiment was conducted in triplicate to validate the experimental errors.

3. Results and discussion

3.1. HPLC/UV procedure

In order to avoid the pressure change, the isocratic mode of mobile phase is applied throughout this work. Fixing the flowrate at 1 ml/min., various mixing ratios of bi-distilled water / acetonitrile (90% ÷ 10%), 10mM phosphate buffer pH 3.5 / acetonitrile (10% ÷ 30%) do not result in any peaks of TMP even at prolonged measuring time, despite its success using gradient mode [e.g. 14,15]. However, mixtures of 0.1% formic acid / acetonitrile (70% ÷ 90%) work relatively well. Taking into account effects of the mobile phase flowrate (1.0 ml/min ÷ 0.25 ml/min) onto the retention time, peak area and – symmetry, the mobile phase composition is chosen 0.1% formic acide / acetonitrile = 82% / 18% (v/v) and its flowrate 0.5 ml/min. TMP signals are the highest at wavelength 270 nm instead of 275 nm as stated in [15]. Figure 2 and Figure 3 show chromatograms of the most dilute TMP samples which are measured in this work, and the constructed calibration line using the HPLC parameters mentioned above. One can see a linear relationship between the TMP peak areas and the corresponding TMP concentrations up to 100 µM with confidence coefficient of 0.9999.

The reproducibility of TMP retention times is pretty good, e.g. t_r = (3.42 ± 0.02) min. results from 12 measurements presented in Figure 2 (triplicate measurement each sample). Certainly, this retention time increases with increasing the 0.1% formic acid / acetonitrile in the mobile phase, e.g. to ~ 90/10. However, the peak shape and symmetry suffer a lot. The TMP peak areas are well reproducible, too. The estimated relative errors are within 3%, even for the most dilute sample (0.2 µM TMP). For the sake of our further application, 0.2 µM TMP is considered the real limit of quantitation (LOQ) and therefore 0.067 µM TMP comes out as the corresponding limit of detection (LOD) of this analytical procedure.

It is worth noting this analytical procedure does not aim to analyze TMP concentration in surface water samples, which are at least about 100x lower than 0.2 µM [14,15]. In such cases, an additional pre-concentration step, e.g. by solid phase extraction (SPE), is necessary. However, it confirms that the calculated removal yields up to 99% even from the initial concentration 20 µM TMP (see below) are reliably determined.

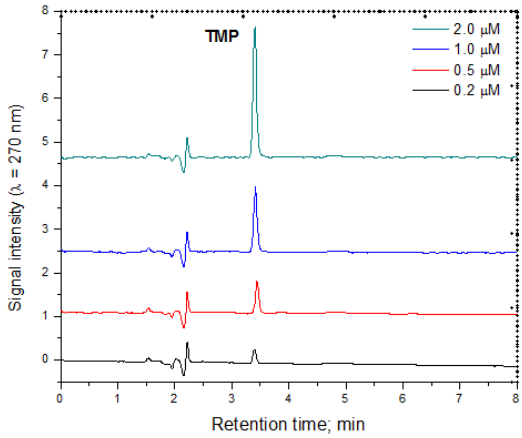

Figure 2. *Chromatograms of dilute TMP samples **

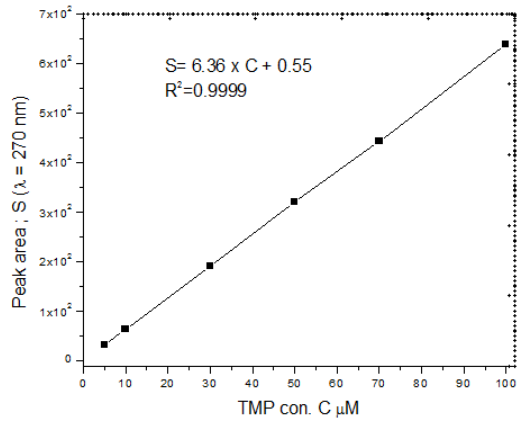

Figure 3. *Calibration line for TMP analysis*

** Mobile phase: 0.1% formic acid / acetonitrile = 82 / 18 (v/v), 0.5 ml/min. Detector wavelength 270 nm, injection volume 10 µl*

3.2. TMP removal yields, -rates, and radio-lytic products

Based on the constructed calibration line in Figure 3, TMP removal yields R_D% are calculated according to the formula:

$$R_D\,\% = \frac{C_0 - C_D}{C_0} = \frac{S_0 - S_D}{S_0}$$

Where symbols C and S refer to TMP concentrations and peak areas, indexes 0 and D refer to samples before and after absorbing dose D, respectively. The sample with initial TMP concentration 140 µM is diluted before analysis and the measured peak area is re-calculated.

Figure 4. shows the TMP removal yields and –rates due to the absorbed doses. Most quantitative removal of the initial TMP concentrations 20 µM, 50 µM, 70 µM, 100 µM, 140 µM is achieved at absorbed doses 0.3 kGy, 1.0 kGy, 1.5 kGy and 3.0 kGy, respectively. As C_0 = 70 µM is comparable with 20 mg/l (~ 69 µM) in the literature [15], our determined dose for a practically

quantitative TMP removal is slightly higher (1.5 vs. 1 kGy). The origins of this difference might be, but not limited to the difference in dose rates of the ^{60}Co sources. Except for the lowest conc. $C_0 = 20$ µM, the experimental results show that TMP removal rates fit well to kinetic equations of pseudo-first order reactions, with the reaction rate depending on the initial TMP concentration. This finding is frequently reported in the literatures [e.g. 16, 17]. In fact, from the theoretical point of view reactions between substrate molecules – in this case TMP molecules – and hydroxyl radicals in irradiated samples are of second order [e.g. 14]. Anyway, these results demonstrate the potential of γ-irradiation as an alternative treatment method for TMP contaminating water. Even for such a high level of contamination as ~ 140 µM TMP, an absorbed dose just about 3.0 kGy is sufficient for its almost quantitative removal.

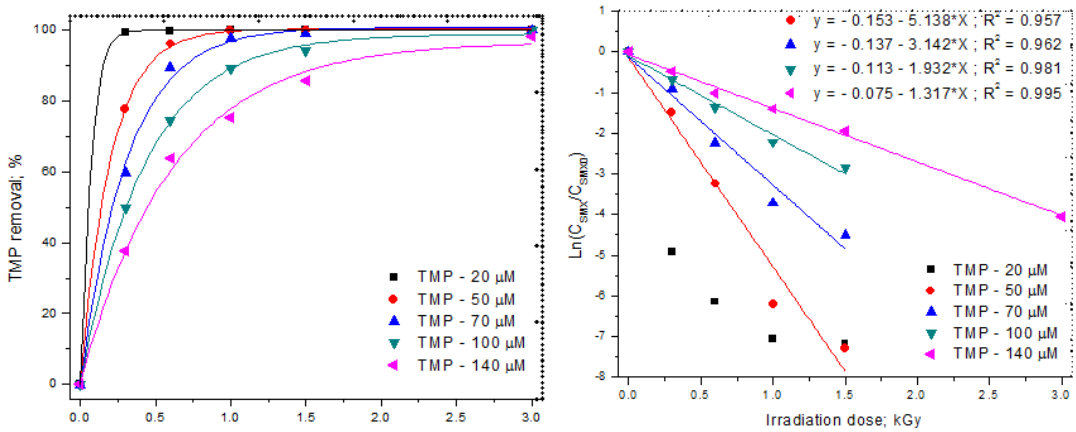

Figure 4. *TMP removal yields (upper) and –rates (lower) depending on its init. conc. and doses*

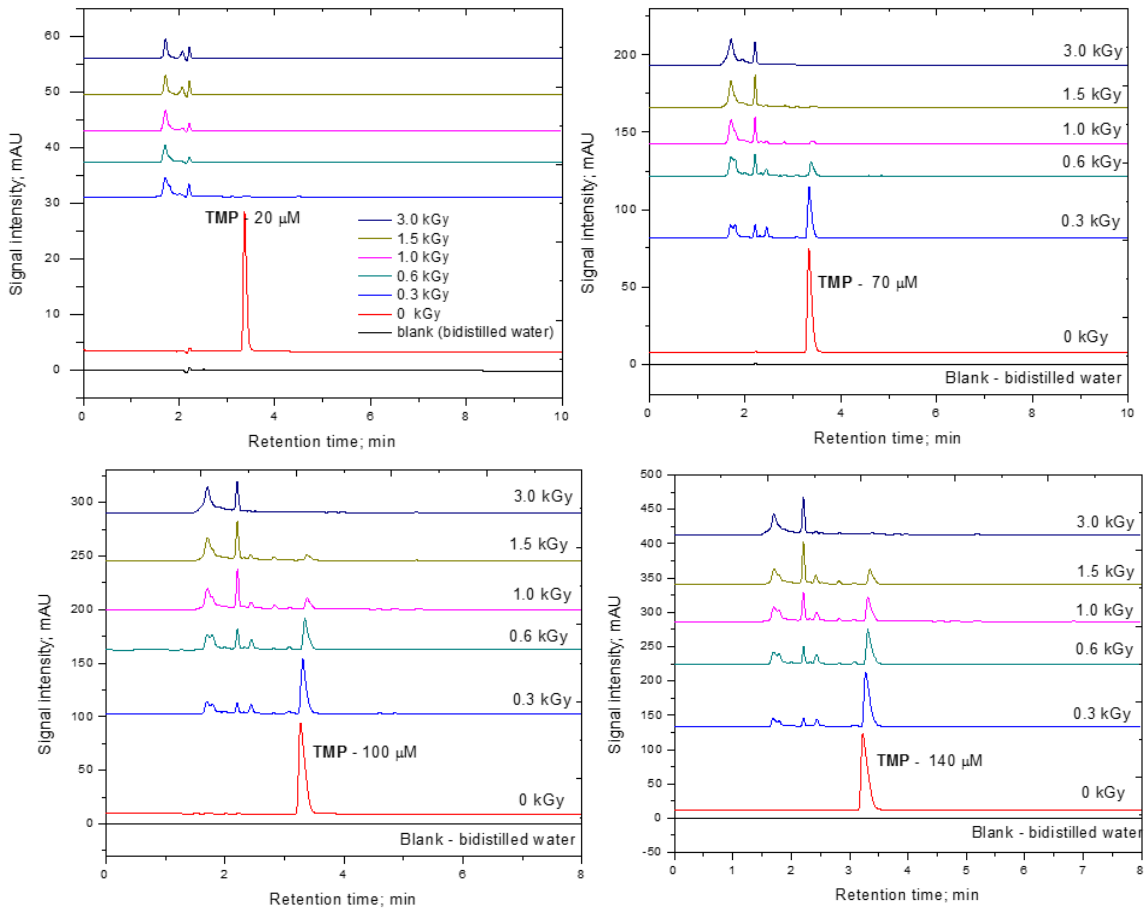

Figure 5. *Chromatograms of TMP samples depending on its initial concentrations and absorbed doses*

Beside the removal yields, the toxicity or even identity of the treatment products has recently become important factors from both theoretical and practical points of view. Normally, sophisticated equipment such as liquid chromatography – time of flight mass spectrometry (LC-TOF-MS) or conventional LC-MS are required [e.g. 14-16]. However, the HPLC-UV chromatograms reveal some characteristics of the treatment products which

absorb UV lights [e.g. 17]. Figure 5 shows up to 6 peaks of TMP radio-lytic products which have retention times shorter than TMP itself. According to the principle of reverse-phase chromatography all these detected UV-absorbing products have higher polarity than TMP. These peaks gradually diminish with increasing the absorbed dose, except for two with the shortest retention times, suggesting that only the corresponding products are stable under γ-irradiation. In addition, comparing chromatograms on Figure 5 and Figure 2 would suggest that these remaining 2 peaks represent the inorganic products. It is in good accordance with the reported ~ 20% TMP mineralized under comparable conditions [15]. However, nothing more could be stated and, moreover, the number of detected peaks are lower than the number of TMP radio-lytic products reported in the literature [14, 15].

It is well known from the literature that in irradiated aqueous samples, water is first radio-lysed to produce many chemically active species including hydroxyl radical $\cdot OH$ – strong oxidant and $\cdot H, e^-_{aqua}$ – strong reductants, which further attack the substrate molecules [e.g. 14, 15, 18].

Under the experimental conditions prevailing in this work, mainly hydroxyl radical is responsible for TMP radiolysis and it is believed to preferentially attack the trimethoxybenzene moiety (TMB), as illustrated in Figure 6, resulting in up to 5 products. They all contain aromatic rings [14, 15] and therefore should be able to absorb UV radiations, too.

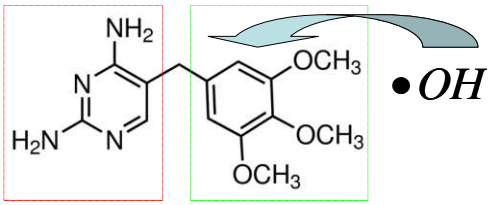

Figure 6. *Preferential attack of* $\cdot OH$ *to TMP*

It is questionable whether some of the TMP radio-lytic products identified by LC-MS in the literature could be ascribed to the peaks mentioned above in the HPLC/UV chromatograms. As indirect evidence, the octanol / water distribution coefficients of the detected products and their precursor – trimethropim – could be accessed by means of computational chemistry and compared with each other [16].

4. Conclusions and outlooks

A suitable HPLC/UV procedure for rapid TMP analysis in aqueous samples is described in details, which enables us to analyze samples in the concentration range of 0.2μM ÷ 100μM TMP. γ-irradiation proves to be an efficient alternative method for treatment of TMP residues in water, as an absorbed dose about of 0.3kGy should be sufficient to quantitatively remove TMP residues at all contamination levels typically found in wastewater effluents and natural aquifers. Further investigation is required to identify/ quantify the radio-lytic products of TMP and/or to compare the toxicity of samples before and after irradiation.

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