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Radiation removal of synthetic estrogens in aqueous solution: influence of reduction or oxidation system and toxicity test

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Abstract Estrogens as a kind of steroidal sex hormone are widely used in humans, especially quinestrol (QS), dienestrol (DS) and norethindrone (NET, 19-nor-17-alpha-ethinltestoster-one), which cannot be completely degraded after application. Steroidal estrogens at low concentration pulling into environment can disturb the normal biological function of wide life and thus lead to great threat to humans. So it is important to explore its degradation mechanism and its behavior in the environment. In this study, we investigated the oxidation or reduction system under gamma irradiation for reducing estrogenic activity in the aqueous solutions as well as degradation kinetics, its by-products and yield of transformation by different analytical methods such as GC–MS and HPLC. Gamma irradiation could effectively degrade estrogens in aqueous solution. The degradation reaction of estrogens could be depicted by first-order reaction kinetics. The total organic carbon of solution decreased with an increasing absorbed dose with the order: quinestrol > norethindrone > dienestrol. The toxicity of the three estrogens was declined after irradiation. Mono- and quadric-hydroxylated intermediates as well as organic acids were formed after gamma irradiation.

Keywords Dienestrol · Gamma irradiation · Norethindrone · Quinestrol · Synthetic estrogens

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1 Introduction

A large volume of steroidal sex hormones (estrogens, progestins and androgens) are used for treatment of menopause and osteoporosis in humans [1, 2]. Most steroidal sex hormones are not completely degraded after their applications [3]. As a result, the steroidal sex hormones and its metabolites enter the ecosystem [4, 5]. The presence of steroidal sex hormones has been detected in effluents from sewage treatment plants and surface waters, often in the low nanograms per liter range [6, 7]. A nationwide survey in China revealed a widespread occurrence of estrogens in source water, containing bisphenol A of 512 ng/L and nonylphenol of 918 ng/L [8]. Steroidal sex hormones with hormonal activity disturb normal biological function of wildlife and thus pose a potential harm to the ecosystem and eventually human health [5], e.g., they may disrupt the endocrine systems of fish at concentrations of <1 ng/L [9]. Based on observed concentrations and estrogenic activities of steroidal sex hormones in effluents, steroidal sex hormones, represented by quinestrol (QS), norethindrone (NET; 19-nor-17-alpha-ethinltestoster-one) and dienestrol (DS), are important synthetic estrogens and attract considerable attention.

QS, DS and NET are synthetic estrogens used in contraceptive and hormone replacement therapy and in treating breast cancer and prostate cancer occasionally [2, 10]. They are designed to resist structural changes to enhance their persistence in the body. QS, DS and NET are discharged into environment through the urine [11]. Synthetic estrogens are more stable than natural estrogens in aqueous solution, hence their greater estrogenic potency than natural estrogens [12, 13]. So the environmental fate of the three compounds becomes a pressing problem and calls for

treatment of the estrogens in water, which become extremely necessary and significant.

A series of physical, biological and chemical methods have been used to remove synthetic estrogen. The presence of estrogenic compounds in effluents from wastewater treatment plants indicates that physical and biological treatments are not effective in removing these pollutants [14]. Chemical methods, advanced oxidation processes (AOPs), have emerged as an effective method to remove non-degradable estrogenic compounds [15–17]. Among AOPs, γ-ray treatment shows high removal efficiency because hydrated electrons (e^-_{aq}), hydroxyl radicals ($^{\cdot}OH$) and hydrogen atoms ($^{\cdot}H$) generated by γ-rays play an important role in degrading toxic organic compounds [18, 19]. While studies have demonstrated degradation pathways of organic compounds related to oxidation or reduction system [20, 21], the degradation kinetics and mechanism of QS, DS and NET under gamma irradiation have not been reported. In this study, we irradiated QS, DS and NET to study their degradation kinetics, so as to develop predictive models for reducing effluent estrogen concentrations down to their detection range of 1.0 ng/L [6, 7]. On the other hand, biological rotifers have been used to study estrogens or endocrine disruptors in the aquatic environment [22–24], so we explored the effect of estrogens and endocrine disruptors via rotifer experiment in this study.

In this paper, we investigate fundamental parameters of oxidation or reduction system under gamma irradiation for reducing estrogenic activity of QS, DS and NET in the aqueous solution. The gamma-ray degradation kinetics is evaluated. The degradation products and their toxicity are studied using GC–MS and high-performance liquid chromatography (HPLC).

2 Experimental

2.1 Materials and methods

Properties of QS, DS and NET are given in Table 1, and their molecular structures are shown in the left of Fig. 1. Quinestrol (99 %, SIGMA, USA), norethindrone (98 %, Toronto Research Chemicals Inc.), and dienestrol (96.1 %, Laboratories of Dr. Ehrenstorfer, Germany), and the organic solvents of pyridine, hexanes, acetonitrile, isopropyl alcohol and methanol applied for HPLC, were purchased from Shanghai ANPEL Scientific Instrument Co., Ltd., China. The solutions were prepared with ultrapure water (18.2 MΩ cm) produced by a Milli-Q water purification system (Millipore, USA).

2.2 Irradiation

Estrogens solutions at an initial concentration of 2 mg/L (10 mL) were prepared in glass dish (Φ70 mm × 35 mm) with plugs, at initial pH values of 6.0–7.5 without adjustment. The samples were irradiated to 0.5–6 kGy in a ^{60}Co source in Shanghai Institute of Applied Physics, Chinese Academy of Sciences. All samples were filtered with 0.22-mm micro-filtration membrane before analysis. All experiments were performed at ambient temperature. The analysis processes are shown in Fig. 1.

2.3 Toxicity tests

The toxicity was determined by rotifer test. Four rotifers were added to 8 cm^3 of test solution in Φ16 mm × 150 mm disposable glass test tubes. The tubes, at 25 °C, were placed on a rotator turning at 10–15 rph. After 2 days, the total numbers of rotifers were counted in each tube. Each sample had three parallel samples. From the data, the rate of population growth (r) was calculated by $r = (\ln N_t - \ln N_0)/2$, where N_t is the number of female rotifers in the tube after 2 days and N_0 is the initial number of rotifers in each tube.

2.4 Analytical methods

The QS, DS and NET samples were quantified with an HPLC-1200 (Agilent, USA), equipped with a C_{18} column (4.6 mm × 250 mm × 5 μm, XDB-18, Eclipse, USA), reverse-phase column and a UV detector of 220 nm, 245 nm and 230 nm in detection wavelength. The column temperature was 30 °C, and the injection volume was 10 μL. All mobile phases were filtered using a 0.45-μm Millipore filter and were degassed by ultrasonication before use. The mobile phase of QS and NET is a mixture of degassed acetonitrile and water at pH 7(80:20, V/V). The mobile phase of DS is a mixture of degassed methanol and water at pH 7 (66:34, V/V). The flow rate was 0.8 cm^3/min. The retention time of QS, DS and NET was 4.74 min, 5.24 min and 2.45 min, respectively.

Table 1 Summary of estrogens used in the study

Estrogens	Melting point (°C)	Boiling point (°C)	Molecular weight
Quinestrol ($C_{25}H_{32}O_2$)	106–112	502.5	364
Dienestrol ($C_{18}H_{18}O_2$)	228–233	231–234	266
Norethindrone ($C_{20}H_{26}O_2$)	205–206	447	298

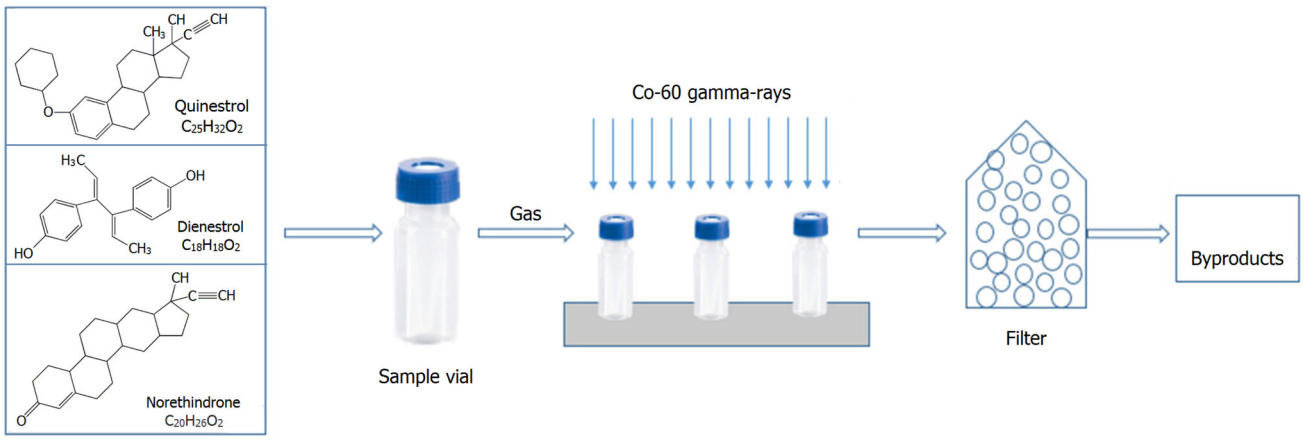

Fig. 1 Experimental processes

Solid-phase micro-extraction with GC/MS was used to analyze the by-products. The irradiated samples of 10 mL were added to the 15 mL vials containing a magnetic stir rotor. The vials, sealed with Teflon septum, were placed on the magnetic stirring stage. They were kept at 50 °C for 20 min and extracted at 200 rpm/min. After extraction, the fiber was removed from the vial and inserted into the injection port of GC/MS system for thermal desorption and analysis. Then, the fiber was removed from the injection port. Before and after the sample desorption, the fiber was inserted into the injection port of GC/MS system for blank sample analysis. Extraction of each sample was repeated three times. GC/MS analyses were performed on a 7890A gas chromatography instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with a mass spectrometer detector MSD 5975C and 7693 automatic liquid sampler. The separation of QS, DS, NET and the irradiation products was carried out on a DB-5MS fused silica capillary column (30 mm × 0.25 mm i.d. × 0.25 µm, J&W Scientific, Agilent, USA.) with the following oven temperature program: initial temperature of 40 °C for 1 min, heated to 180 °C at a rate of 8 °C/min, to 310 °C at a rate of 15 °C/min, and kept for 10 min. Helium was used as the carrier gas, at the flow rate of 0.8 dm^3/min. Injector temperature was maintained at 250 °C, and the injection volume was 10 µdm^3. The mass spectrometer was operated at a selected ion monitoring (SIM) mode for QS (*m*/*z* 364), DS (*m*/*z* 266), NET (*m*/*z* 294), and scan mode *m*/*z* in the range of 50–450 for the by-products, respectively.

The pH and *TOC* values were measured by a pH meter (Dual Star, Thermo Inc., USA) and a *TOC* analyzer.

2.5 Quality assurance and quality control

Field blanks ($n = 8$) were analyzed with every batch of samples against contaminations or interferences. Sample concentrations were determined from external calibration curves prepared at concentrations ranging from 0.5 to 1000 ng/mL for QS, DS and NET. Strong coefficients of 0.9998, 0.9999 and 0.9994 for QS, DS and NET, respectively, were determined. No interferences were detected in the field blanks.

2.6 First-order kinetic model of estrogen gamma irradiation degradation

The following pseudo-first-order gamma irradiation degradation kinetic model was assumed to relate changes in the total estrogen concentration in batch tests [25].

$$C = C_0 e^{-dD}, \tag{1}$$

$$-\ln(C/C_0) = dD, \tag{2}$$

where C_0 and C are concentrations of the pollutant before and after irradiation, respectively; d is the dose constant in units of a reciprocal dose; and D is absorbed dose. Knowing d, the first-order rate law, Eq. (1), can be used to calculate the dose requirement (D) to achieve desired decreases in pollutant concentrations. The dose constant can be used in certain investigations into the fundamentals of radiolysis reaction mechanisms. In some cases, it can be deemed as a dose-dependent pseudo-first-order "rate constant" [26].

2.7 Estrogens radiation chemical yield (*G*-value)

Radiation chemical yield (defined by the number of molecules formed or destroyed in solutions absorbing 100 eV of radiation energy) can be calculated using Eq. (3) [27–29],

$$G = \Delta R N_A / (D \times 6.24 \times 10^{16}), \tag{3}$$

where ΔR is the amount of reduced estrogens (mol/L); $N_A = 6.023 \times 10^{23}$ (molecules/mol) is Avogadro

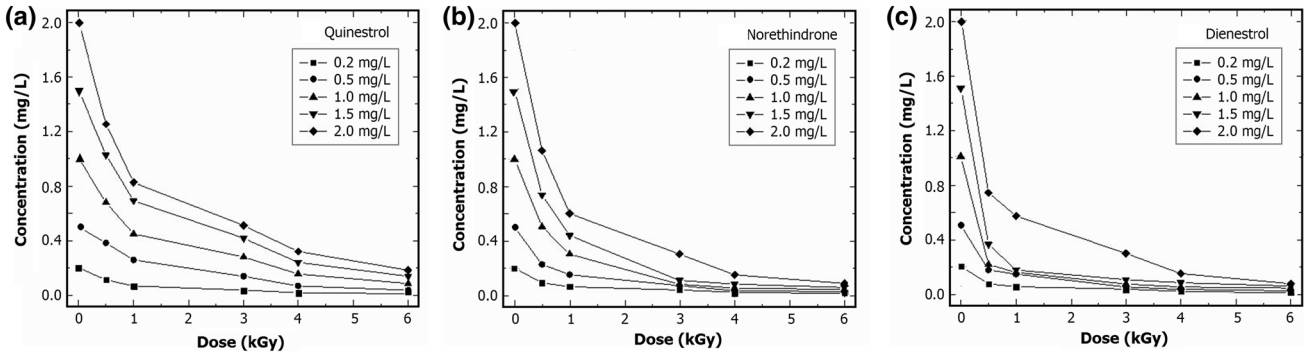

Fig. 2 Degradation of QS, DS and NET estrogens irradiated to different doses

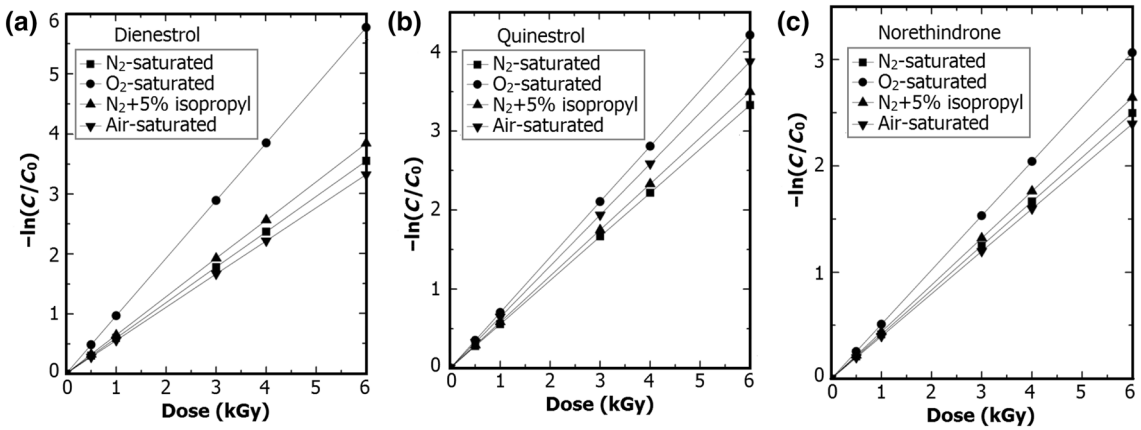

Fig. 3 Degradation kinetics of QS, DS and NET estrogens irradiated in different systems

Table 2 *G* value of the estrogens irradiated in O_2 gas

Dose (kGy)	0.5	1	3	4	6
DS ($\times 10^{-2}$)	8.30	4.87	1.79	1.38	0.93
QS ($\times 10^{-2}$)	6.78	4.27	1.79	1.52	1.07
NET ($\times 10^{-2}$)	9.47	5.03	1.94	1.59	1.10

constant; *D* is the absorbed dose (Gy); and 6.24×10^{16} is the conversion coefficient from Gy to 100 eV/L.

3 Results and discussion

3.1 Degradation kinetics of estrogens

Radiation-induced degradation of organic compounds was initiated by primary species produced from water radiolysis, as given in Eq. (4). In neutral solutions, OH, e^-_{aq} and H are major primary species. In the presence of dissolved oxygen or air, the reducing species, H and e^-_{aq} can be converted into oxidizing species: perhydroxyl radicals (HO_2) and super oxide radical anions (O_2), as given in Eqs. (5) and (6) [30–32]. Thus, in the presence of air, OH is considered to be significant oxidizing transient for estrogens degradation. Oxidizing radicals, especially $^{\cdot}$OH radicals, attack double bonds (C–C in estrogens molecular), forming OH^- adducts. The carbon atom can be attacked by $^{\cdot}$OH radicals, leading to the cleavage of C–C bond, producing some intermediates. The OH^-adducts can easily scavenge oxygen to form the corresponding unstable peroxyl radicals. Hydrolysis, radical–radical recombination or other reactions can take place, forming various final products [33, 34]. In the presence of air, reduction system occurs during gamma irradiation, as given in Eqs. (7) and (9).

$$H_2O \rightarrow e^-_{aq}(2.6) + H^{\cdot}(0.55) + {}^{\cdot}OH(2.7) + H_2(0.45) + H_2O_2(0.71) + H_3O^+(2.6), \quad (4)$$

$$H^{\cdot} + O_2 \rightleftarrows HO^{\cdot}_2 \quad K = 2.1 \times 10^{10}\,\mathrm{L\,mol^{-1}\,s^{-1}}, \quad (5)$$

$$e^-_{aq} + O_2 \rightleftarrows O^{\cdot 2-} \quad K = 1.9 \times 10^{10}\,\mathrm{L\,mol^{-1}\,s^{-1}}, \quad (6)$$

$$HO^{\cdot}_2 + O^{\cdot 2-} \rightleftarrows H_2O_2 + O_2 (PH < 7) \quad K = 9.7 \times 10^{7}\,\mathrm{L\,mol^{-1}\,s^{-1}}, \quad (7)$$

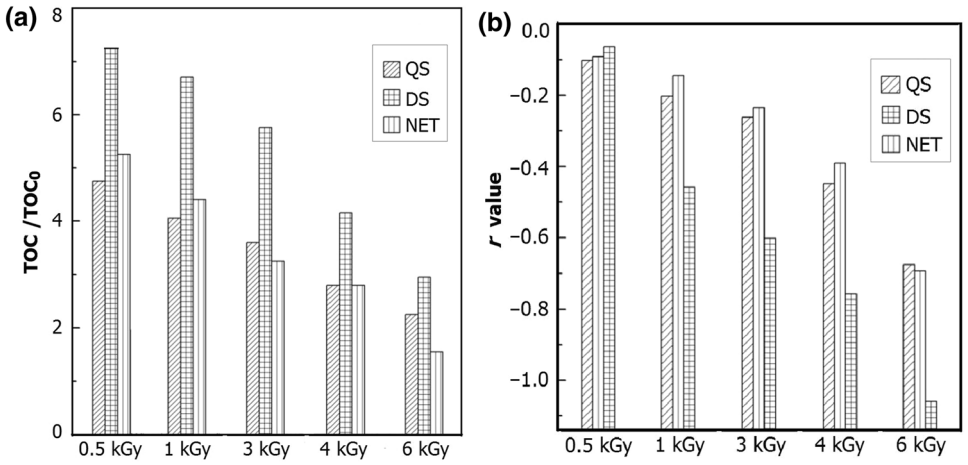

Fig. 4 *TOC* (**a**) and *r* (**b**) values of QS, DS and NET irradiated to different doses in O_2 atmosphere

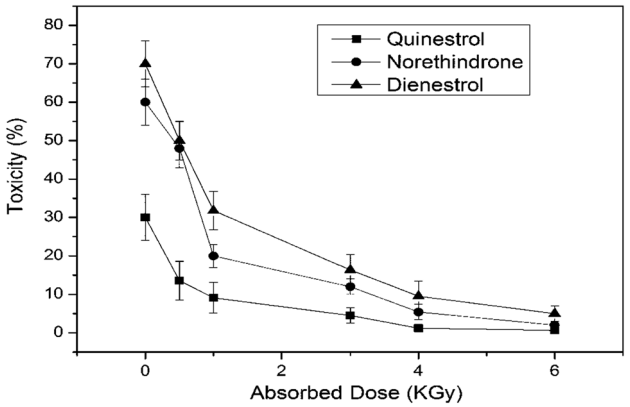

Fig. 5 Toxicity of the irradiated NET, QS and DS

$$HO_2^{\cdot} + HO_2^{\cdot} \rightleftarrows {}_2 + O_2 \quad K = 8.3 \times 10^5\,\mathrm{L\,mol^{-1}\,s^{-1}}, \qquad (8)$$

$$H^{\cdot} + OH^- \rightleftarrows e_{aq}^- + H_2O \quad K = 2.2 \times 10^7\,\mathrm{L\,mol^{-1}\,s^{-1}}. \qquad (9)$$

The estrogens in aqueous solution were irradiated to 0.5 kGy, 1.0 kGy, 3.0 kGy, 4.0 kGy and 6.0 kGy by ^{60}Co γ-rays. The concentrations of QS, DS and NET varying with doses are shown in Fig. 2. The estrogen concentrations decreased with increasing doses. It changed slowly after 4 kGy to reach the degradation efficiency of about 100 % at 6 kGy.

Radiation-induced degradation of estrogens in aqueous solution of different systems is shown in Fig. 3. The estrogens were degraded rapidly with increasing doses. In N_2-saturated aqueous solutions with 5 % isopropanol, the $HO^{\cdot}$ radicals are scavenged by isopropanol. Therefore, the short-lived species reacting with estrogens is e_{aq}^-. The degradation of estrogens was at reduction system. The degradation rate of estrogens increased with dose. In O_2-saturated aqueous solutions, most of the electrons and H atoms react with oxygen. Therefore, the short-lived species reacting with estrogens is $HO^{\cdot}$. The estrogens removal in O_2-saturated aqueous solutions was the highest of all conditions.

3.2 *G* values

According to Eq. (3), the *G* values of estrogens (DS, QS and NET, 2 mg/L each) at different doses in oxygen gas are given in Table 2. The *G* values decreased with the increasing doses. This may due to the competitive reactions of radicals between the parent compounds and degradation products, or the recombination of radical–radical ($^{\cdot}OH$, e_{aq}^- and $H^{\cdot}$), so the radical concentrations for reaction with estrogens were reduced. The trend of radiation chemical yield is consistent with published findings for radiolytic degradation of malathion and lindane [35].

3.3 Removal of *TOC* and *r* values

TOC values of the radiolytic products in O_2 atmosphere were examined to evaluate the effect of mineralization of DS, QS and NET estrogens at different doses (Fig. 4a), with their initial concentration of 2 mg/L. The *TOC* at 0.5 kGy was the highest, decreasing with increasing doses. Below 3 kGy, *TOC* values decreased slowly, while above 3 kGy, it decreased quickly. This is due to that irradiation products of DS, QS and NET remained in the solutions without being mineralized at lower absorbed doses. The order of *TOC* is QS > NET > DS.

It was reported that parent estrogens and some of the intermediate products of pollutants can disturb the normal physiological response and lead to sex steroid imbalance [36]. Hence, effect of the intermediate products of pollutants should be tested before discharging them into the ecosystem. In the irradiated solutions, the negative effect decreased with increasing doses (Fig. 4b). It is obvious that even at the start of irradiation the *r* value of the reactive

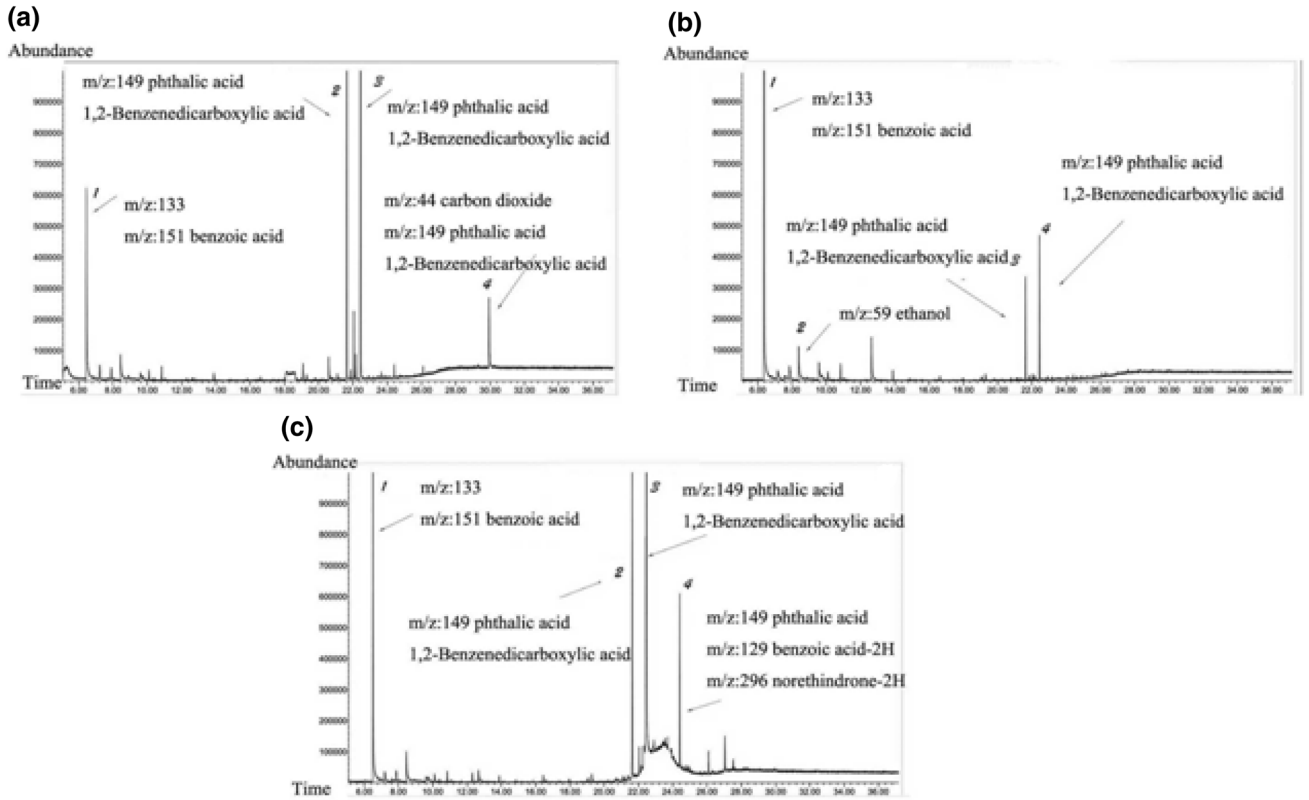

Fig. 6 TIC of estrogens from GC–MS a–d: the main *m/z* after gamma irradiation. (**a**) Quinestrol. (**b**) Dienestrol. (**c**) Norethindrone

intermediates decreased. The order of *r* value is QS > NET > DS. We think that the rate of degradation was affected by the amount of benzene contained in the estrogens. Quinestrol can be completely mineralized under γ-irradiation. The population of QS is low.

3.4 Toxicity analysis

Rotifers can be used as biomarkers for the presence of endocrine-disrupting chemicals in the environment [22, 23]. Thus, we attempted to explore the toxicity of QS, DS and NET with rotifers before and after their irradiation.

As shown in Fig. 5, toxicity of the three irradiated estrogens declined with increasing doses, decreased rapidly by 1 kGy irradiation, and changed slowly from 1 to 6 kGy, especially from 4 to 6 kGy. The order of toxicity values of the irradiated QS, DS and NET is DS > NET > QS. These indicate that the estrogens in the environment can affect the normal biological reproduction, which were connected with other study about estrogens or endocrine disruptors [22–24].

3.5 Estrogens derivatives from γ-irradiation process

In order to identify the QS, DS and NET derivatives, samples irradiated to 1 kGy were analyzed by GC–MS. The derivatives and their mass spectrums are shown in Fig. 6.

It was observed that an attack of hydroxyl radicals on the propanoic acid and isobutyl substituents of QS, DS and NET structures results in the formation of products such as phthalic acid, 1,2-benzenedicarboxylic acid and benzoic acid. In Fig. 6, the peaks at $m/z = 133$ and $m/z = 151$ indicate benzoic acid and $m/z = 149$ shows the formation of phthalic acid and 1,2-benzenedicarboxylic acid. In Fig. 6a, the peak at $m/z = 149$ is from phthalic acid, the peak at $m/z = 44$ is from carbon dioxide, and molecular weight 364 is quinestrol. In Fig. 6c, the peak at $m/z = 44$ is carbon dioxide, the peak at $m/z = 129$ is benzoic acid and $m/z = 296$ decreased 2H from norethindrone [37]. Tao Tang et al. [10] reported the formation of products estrone, organic acid and carbon dioxide, in their study of UV degradation of QS. Dai et al. [38] argued that the hydroxylation process could be the first step of the degradation, followed by a second step of demethylation or decarboxylation with other different by-products with smaller *m/z* values [3]. Tang et al. [10] and Chowdhury et al. [39] reported the formation of products benzeneacetic acid and phenylacetic acid by the UV/sonolysis degradation of quinestrol. However, Huber et al. [40] and Kurisu et al. [41] suggested that the final photodegradation products were acid derivatives.

4 Conclusion

Gamma irradiation can effectively degrade estrogens in aqueous solution, with degradation efficiency of 100 % at 6 kGy. The degradation reaction of estrogens can be depicted by first-order reaction kinetics. Different atmosphere conditions can alter the degradation efficiency. The addition of isopropyl alcohol also influences the estrogens degradation process. *TOC*s of the irradiated estrogen solutions, decreased with increasing doses, are in the order of quinestrol > norethindrone > dienestrol. Toxicity of the three irradiated estrogens declined with increasing doses. HPLC and GC–MS were used for identifying the derivatives. Gamma irradiation of estrogens leads to the formation of its mono- and quadric-hydroxylated intermediates, and products like phthalic acid and benzenedicarboxylic acid due to the oxidation of propanoic acid and isobutyl substituents of the estrogens. The by-products can disturb the normal physiological response.

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