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Chemical analysis applied to the radiation sterilization of solid ketoprofen

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The aim of this work is to investigate the feasibility of radiation sterilization of ketoprofen from a chemical point of view. Although irradiated ketoprofen has already been studied in the literature [Katušin-Ražem *et al.*, Radiat. Phys. Chem. 73 111–116 (2005)], new results, on the basis of electron spin resonance (ESR) measurements and the use of hyphenated techniques (GC-MS and LC-MS), are obtained. The ESR spectra of irradiated ketoprofen consists of four unresolved resonance peaks and the mean G-value of ketoprofen is found to be 4 ± 0.9 nmoles/J, which is very small. HPLC-UV analyses indicate that no significant loss of ketoprofen is detected after irradiation. LC-MS-MS analyses show that the structures of the non-volatile final products are similar to ketoprofen. Benzaldehyde is detected in the irradiated samples after dynamic-extraction GC-MS. The analyses show that ketoprofen is radioresistant and therefore might be radiosterilized.

Keywords: Ketoprofen; Radiation sterilization; ESR; Radiolytic products

1. Introduction

Ketoprofen is a non-steroidal anti-inflammatory drug [1]. Its intravenous form is widely prescribed in human and veterinary medicine. The chemical structure of ketoprofen is presented in figure 1. This work studies the chemical radioresistance and the feasibility of radiation sterilization of solid ketoprofen.

Radiosterilization is recognized by all major pharmacopeias as an attractive alternative method for the sterilization of solid drugs. Its advantages include terminal sterilization, small temperature rise, low chemical reactivity and dosimetric release [2, 3]. The major problem of radiosterilization are the radiolytic products induced upon irradiation that are generally present in very small quantities (traces) [4].

Electron spin resonance (ESR) spectroscopy appears to be well suited for the determination of the radicals produced after irradiation as well as the detection of irradiated drugs and some irradiated foods [5–7]. Moreover, its sensitivity is very high and it is non-destructive for the sample.

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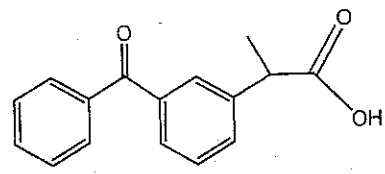

Figure 1. Chemical structure of ketoprofen.

Chromatographic techniques linked to mass spectrometry (LC-MS and GC-MS) allow the detection and the characterization of the radiolytic products which could be very useful in order to understand the radiolytic mechanisms leading to their formation [8, 9].

Hyphenated techniques (LC-MS and GC-MS) and ESR spectroscopy are used as complementary techniques in order to study the effects of ionizing radiations on solid ketoprofen. Although irradiated ketoprofen has already been investigated [10], new results are obtained in this study. ESR is used to characterize free radicals trapped in ketoprofen upon irradiation. The loss of the drug substance (chemical potency) is evaluated by HPLC-UV. The non-volatile and volatile radiolytic products induced by irradiation of ketoprofen are studied by liquid chromatography-electrospray mass spectrometry (LC-ESI-MS) and by gas chromatography-mass spectrometry (GC-MS), respectively.

2. Materials and methods

Ketoprofen is kindly provided from Eczacıbaşı Sağlık Ürünleri Sanayi ve Ticaret A.Ş., Turkey and is stored at room temperature in a sealed container protected from light. The samples are irradiated in sealed glass vials protected from light. Gamma irradiations are performed at room temperature with a panoramic ^{60}Co chamber (UCL, Louvain–La–Neuve, Belgium). The dose rate is 265 Gy/h. The source is calibrated by alanine dosimetry [11]. Samples are irradiated at different doses (15, 25, 40 and 50 kGy).

2.1 *ESR apparatus and conditions*

ESR measurements are made with a Bruker EMX X-band (9.3 GHz) spectrometer. Signal intensities are calculated by the Bruker WinEPR software. The spectrometer operating conditions are as follows. The microwave power is varied from 0.004 to 120 mW for power saturation studies and is set at 1 mW for all quantitative measurements; modulation frequency: 100 kHz; modulation amplitude: 0.1 mT; sweep width: 20 mT. The absolute g-values are determined in comparison with a 1,1-diphenyl-2-picrylhydrazyl (DPPH) reference powder sample ($g = 2.0036$) which is also used to verify the day-to-day response of the spectrometer. The temperature of the sample inside the microwave cavity is monitored with a digital temperature control system BVT 300. The temperature of the samples is decreased from room temperature to 150 K, then increased to 355 K by increments of 10 K and finally decreased to room temperature.

2.2 *HPLC-UV analyses*

The samples are dissolved in methanol (5 mg/ml) and the solutions are injected into an HPLC-UV system composed of a Kontron 422 Pump, a Rheodyne manual injector with a 20 µl loop and a Kontron Capillary-433 UV-visible detector set at 254 nm. The chromatographic separation is performed on a Merck Lichrospher® 100 RP-18e, 250 × 4 mm (5 µm particle

size). The mobile phase consists of 55% (v/v) HPLC-grade methanol and 45% (v/v) water adjusted to pH 2.5 with formic acid. The flow rate is 1 ml/min.

2.3 *LC-MS*

The system consists of a Merck-Hitachi L-6200 Pump, a MIDAS 830 autosampler and a Kontron HPLC 322 UV-visible detector linked to a Themo-Finnigan MAT LCQ® advantage mass detector with an electrospray ionization (ESI) source. The Xcalibur software version 1.3 is used. The capillary temperature is 300 °C, sheath gas is 40 AU, the mass spectrometer is used in the negative ion mode. The chromatographic conditions are the same as in the HPLC-UV analyses and the same solutions are injected.

2.4 *GC-MS*

The dynamic-extraction GC-MS system is composed of an Interscience Thermoquest Trace GC 2000 with a Tekmar Dohrman Velocity XPT accelerated purge and trap sample concentrator linked to an Interscience Trace MS with an electron impact (EI) ionization source. Separation is performed on a CP-Wax 52 CB (25 m × 0.25 mm) column. Helium at a flow rate of 1.9 ml/min is used as the carrier gas. The following conditions are used: injector temperature: 250 °C; the oven temperature is set at 40 °C for 5 min and increased to 240 °C at 5 °C/min; the electron energy is 70 eV, the detector is used in the positive ion mode and full scans from 1 to 420 m/z (mass-to-charge ratio = mass number of an ion divided by its charge) are performed. The peaks are identified in comparison of their mass spectra with those of the NIST (National Institute of Standards and Technology) library. The hypotheses are confirmed by the injection of high-purity standards.

3. Results and discussion

3.1 *ESR experiments*

3.1.1 General features of the ESR spectra. Unirradiated ketoprofen exhibits no ESR signal while irradiated samples show a complex ESR spectrum consisting of four resonance peaks [10]. The spectra recorded at room temperature for gamma-rays-irradiated samples at 25 and 40 kGy are shown in figures 2a and b, respectively. An increase in the absorbed dose does not change the pattern but gives more intense spectra. The most intense resonance line, which is located in the middle of the spectrum, is found to have a g-value of 2.0088 and a peak to peak width of 21.5 G. The lines are broad and superimposed, which leads to badly resolved spectra. The signal-to-noise ratio is low, even for samples irradiated at high doses.

3.1.2 Radicals yield of ketoprofen. To ensure that power saturation does not occur, a microwave power of 1 mW is set during the experiments. The radiolytic radicals yield is determined through the comparison of the ESR signal areas with that of alanine standard measured under similar experimental conditions, the laboratory calibration curve of alanine [11] is used to determine the radical yield. The radical yields for irradiated ketoprofen are 2.9 nmol/J (15 kGy), 3.6 nmol/J (25 kGy) and 4.0 nmol/J (40 kGy). The mean G-value for ketoprofen is 4 ± 0.75 nmol/J which is more than 100 times smaller than the alanine radical yield. This indicates that relatively few radicals are trapped in ketoprofen [10] and therefore, it could be considered as radioresistant [11].

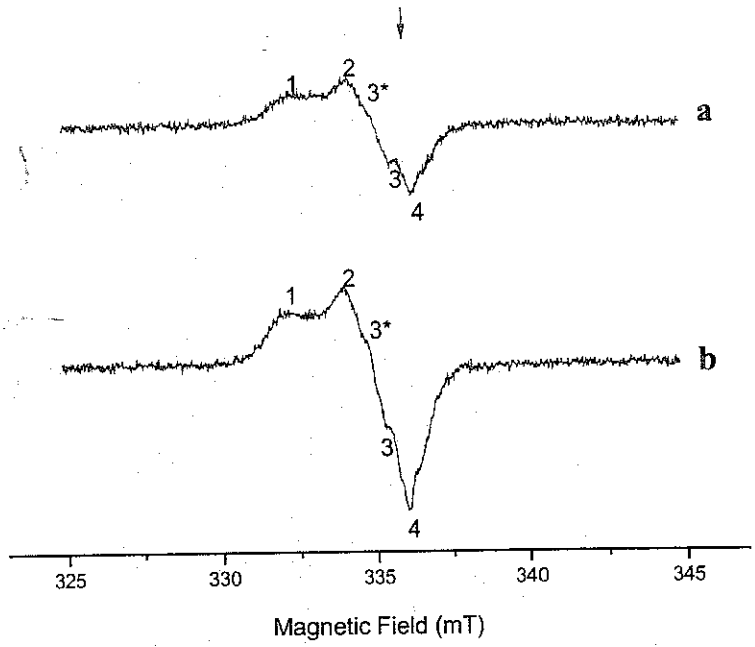

Figure 2. Room temperature ESR spectra of gamma irradiated ketoprofen at 25 kGy (a) and 40 kGy (b). The position of the DPPH line is indicated by the arrow.

3.1.3 Power dependence. Variation of the ESR resonance lines as a function of the applied microwave power (from 0.004 to 120 mW) for solid ketoprofen irradiated at 40 kGy is shown in figure 3. The results indicate that the resonance peaks saturate with different rates. The

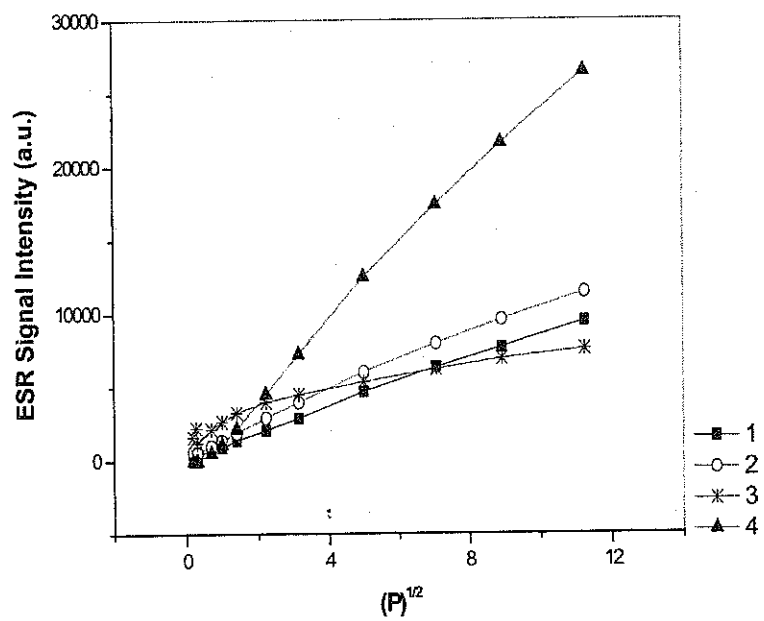

Figure 3. Variations of ESR peak heights versus square root of applied microwave power (P) for solid ketoprofen irradiated at 40 kGy. ■ (line 1), ○ (line 2), ∗ (line 3), ▲ (line 4).

different behaviors of the peaks are an indication that at least two different radical species are present in irradiated ketoprofen which could explain the experimental spectra [4].

3.1.4 Variations of ESR signals with temperature. The variations of the resonance peak heights and intensities with the temperature in the ranges of 294–150 K (figure 4) and 300–355 K (figure 5) are investigated.

Cooling the sample down to room temperature causes a reversible increase in the peak heights and intensities. Warming the sample above room temperature does not produce any significant change up to 320 K. However, an increase of the sample temperature above 320 K causes a continuous decrease in the intensity and the height for all the resonance peaks with the exception of the fourth peak, which becomes dominant at high temperatures. This peak

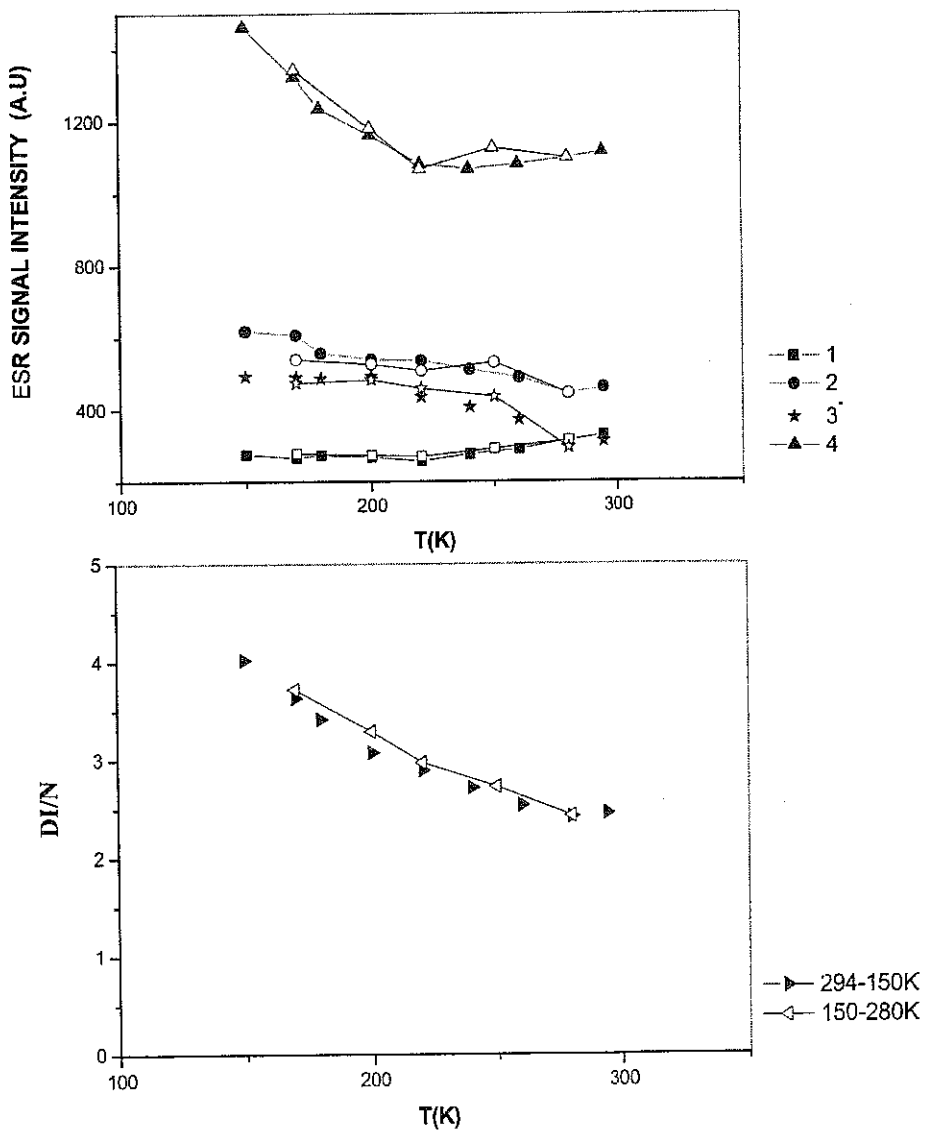

Figure 4. Variation of ESR peak heights and normalized intensities (DI/N) with temperature (294–150 K) for ketoprofen irradiated at 40 kGy. ■ (line 1), ○ (line 2), ★ (line 3), ▲ (line 4).

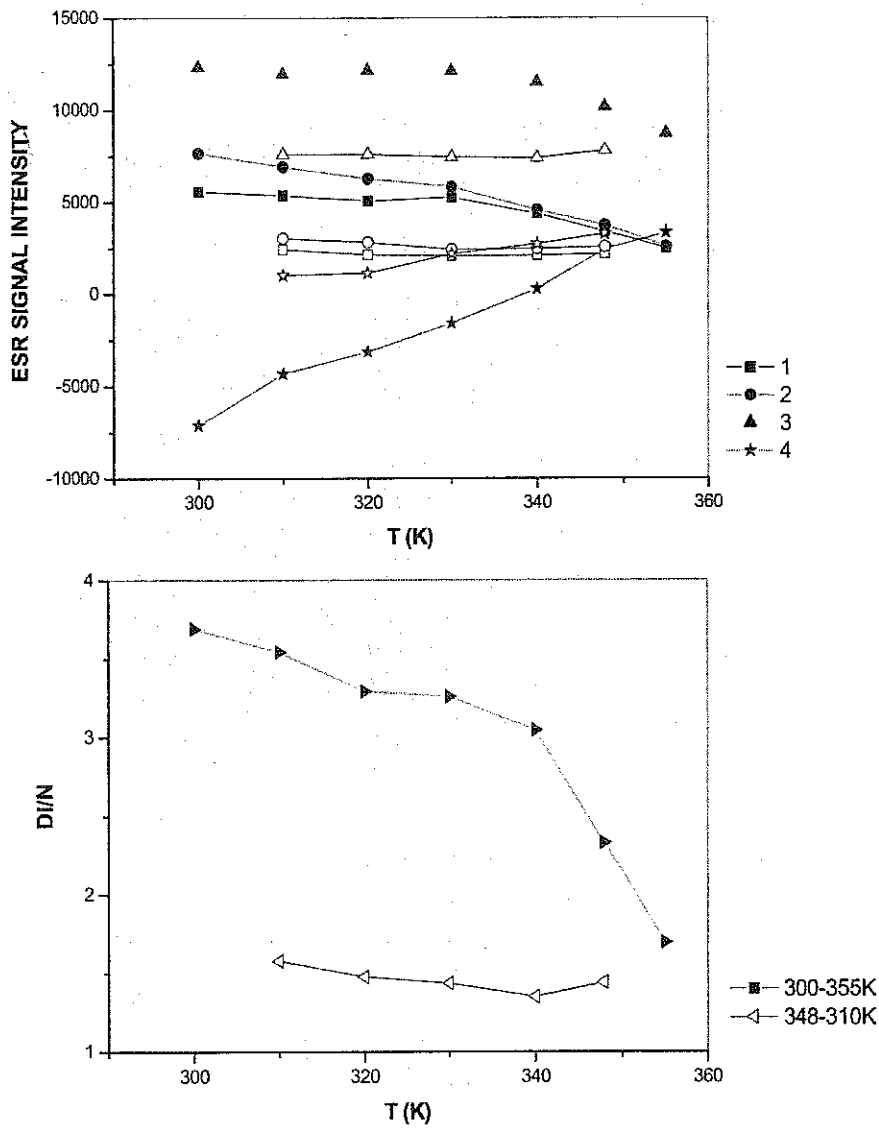

Figure 5. Variation of ESR peak heights and normalized intensities (DI/N) with temperature (300–355 K) for 50 kGy irradiated ketoprofen. ■ (line 1), ○ (line 2), ★ (line 3), ▲ (line 4).

probably belongs to a more stable radical as compared to the other(s). This is another evidence that at least two different radical types are trapped in irradiated solid ketoprofen. The decrease observed at high temperature is irreversible for all peaks, but as the storing conditions for the drugs are generally not higher than 320 K, the radicals can be considered to be stable upon temperature.

3.2 *HPLC-UV analyses*

An overlay of the chromatograms at 254 nm of ketoprofen before and after irradiation at 40 kGy is presented in figure 6. The chromatograms of irradiated ketoprofen show some small peaks with retention times (RT) of 10, 15, 17 and 19 min, respectively. These peaks increase

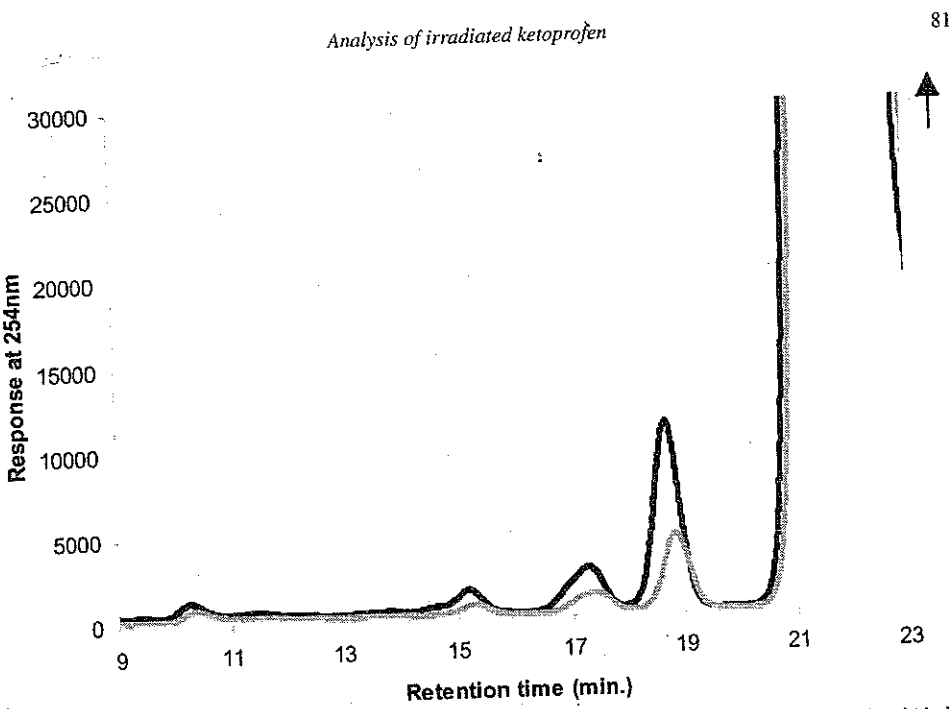

Figure 6. Overlay of chromatograms zoomed on the radiolytic products peaks for 40 kGy gamma-irradiated (darker chromatogram) and unirradiated ketoprofen (lighter chromatogram).

with the absorbed dose and are not unique to radiolysis as they are present in smaller amounts in unirradiated samples.

The areas under the curve of the peak of ketoprofen for irradiated samples are compared with the ones of unirradiated samples. The differences between irradiated and unirradiated samples are not significant, which suggests a very high resistance of ketoprofen toward gamma irradiation. This high resistance can be due to the structure of ketoprofen which has two aromatic rings (see figure 1) that absorb the given energy and redistribute it to the whole molecule instead of one particular bond [11].

3.3 *LC-MS analyses*

In the negative ion mode, pseudomolecular ions corresponding to deprotonated molecules are detected for all the peaks. No co-elution is observed. The peaks are found to have m/z of 269 (RT 10 min), 225 (RT 15 min) and 283 (RT 19 min). For all the radiolytic products and ketoprofen (m/z 253), the fragmentation of the pseudo-molecular ion peak leads to a m/z 209 fragment which could be attributed to the ethyl, 1-(3-benzoyl phenyl) part. This indicates that the structures of the radiolytic products are very similar to ketoprofen as they all have a benzophenone ring. This confirms the resistance of the ring.

3.4 *Dynamic-extraction GC-MS*

In order to detect and identify volatile products that might be generated during the radiolysis of solid ketoprofen, a dynamic extraction GC-MS experiment is performed. In the chromatograms of both irradiated and unirradiated samples, acetaldehyde, ethanol and benzene are detected. They could be residual solvents that originate from the drug synthesis [12]. However, in the chromatograms of irradiated samples, some new peaks are detected and, after comparison

with NIST reference spectra and injection of standard compounds, one of them is attributed to benzaldehyde. This indicates that a breakage of the benzophenone ring might be possible, although no such radiolytic product is observed in the LC-MS analyses.

3.5 *Organoleptic properties*

Colour and odour changes are often observed after radiation treatment [13, 14]. In the case of ketoprofen, no change of color and no smell are detected, even at the highest absorbed dose.

4. Conclusion

Different approaches allowed the characterization of irradiated solid ketoprofen from a chemical point of view. The amount of radicals observed in irradiated ketoprofen is very low and microwave power and temperature studies point out that at least two radicals are present, which confirms the previous observations [10]. After irradiation, no significant loss of ketoprofen is detected by HPLC-UV. LC-MS-MS analyses show that the structures of non-volatile final products are similar to ketoprofen. Some volatile radiolytic products, including benzaldehyde, are detected by dynamic extraction GC-MS.

All the experiments show that solid ketoprofen is very stable toward ionizing irradiation and therefore, radiosterilization might be a suitable method for the sterilization of solid ketoprofen.

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References

[1] B.G. Katzung, *Basic and Clinical Pharmacology, 7th ed.*, (Appleton & Lange, McGraw-Hill, New York, 1998).
[2] B.D. Reid, J. Pharm. Sci. Tech. **49** 83–89 (1995).
[3] A.S. Crucq, V. Deridder and B. Tilquin, Radiat. Phys. Chem. **72** 355–361 (2005).
[4] M. Gibella, A.S. Crucq, B. Tilquin *et al.*, Radiat. Phys. Chem. **58** 69–76 (2000).
[5] T. Miyazaki, T. Kaneko, T. Yoshimura *et al.*, J. Pharm. Sci. **83** 68–71 (1994).
[6] J. Raffi, S. Gelly, P. Piccrelle *et al.*, Rad. Phys. Chem. **63** 705–707 (2002).
[7] Ş. Çolak and M. Korkmaz, Int. J. Pharm. **285** 1–11 (2004).
[8] N. Barbarin, B. Tilquin and E. de Hoffmann, J. Chrom. A **929** 51–61 (2001).
[9] P. Horsch, L. Bigler and H.R. Altorfer, Int. J. Pharm. **222** 205–215 (2001).
[10] B. Katušin-Ražem, K. Hamitouche, N. Matlar-Strmečki *et al.*, Radiat. Phys. Chem. **73** 111–116 (2005).
[11] A. Engalytcheff, R. Debuyst, G.C.A.M. Vanhaelewyn *et al.*, Radiat. Res. **160** 103–109 (2003).
[12] N. Barbarin, A.S. Crucq and B. Tilquin, Radiat. Phys. Chem. **48** 787–794 (1996).
[13] N. Barbarin, B. Rollmann and B. Tilquin, Int. J. Pharm. **178** 203–212 (1999).
[14] M. Gibella and B. Tilquin, Analysis **27** 657–662 (1999).