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Short communication

Radiochemical stability of fluconazole in the solid state

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Abstract

The effect of ionizing radiation in doses between 20 and 200 kGy on physicochemical properties of fluconazole (α-(2,4-diflurophenyl)-α-(1*H*-triazol-1-methyl)-1*H*-1,2,4-triazole-1-ethanol) in the solid state was examined. A number of qualitative and quantitative methods such as scanning electron microscopy (SEM), nuclear magnetic resonance (NMR), ultraviolet (UV) and infrared (IR) spectroscopy, thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC) and organoleptic analysis were used to determine and analyse any changes resulting from irradiation.

A change in colour from white to cream was observed at even smallest dose (20 kGy) and as the dose increased the colour deepened from salmon pink to orange at the highest dose of 200 kGy.

The UV method showed an increase in absorbance at λ_{max} and an appearance of an additional band in the range 280–310 nm for irradiated samples. These changes were associated with the appearance of one to two decomposition products observed by TLC.

Depending on the dose of radiation, the HPLC method detected between 2 and 3 radiolysis products and the decreasing fluconazole content from 0.48 to 7.12%.

The remaining analytical methods (SEM, IR and NMR) did not provide any conclusive information in respect of radiological stability of fluconazole.

The results indicate that fluconazole is a compound of low radiological stability and should not be sterilized using gamma, beta or E-beam radiation.

Keywords: Fluconazole; Radiation sterilization; HPLC; IR; NMR; SEM; TLC; UV

1. Introduction

Fluconazole is a popular antifungal drug belonging to the third generation of azole derivatives. This fluorinated bis-triazole (Fig. 1) is relatively soluble in water and therefore can be administered both orally and intravenously. Fluconazole is particularly successful in treating different types of candiolosis. It also gives good results in treating systemic candiolosis and streptococcal meningities in patients with reduced immunity due to tumours or AIDS [2,3].

In such serious conditions, it is necessary to link fluconazole therapy with other drugs required to treat underlying conditions such as liver or kidney disorders [4–6]. Therefore, it is of particular importance to devise quick, accurate and sensitive analytical methods to determine the level of fluconazole in the blood and body fluids, and the rates of fluconazole clearance. Currently, the most popular method of determination of fluconazole is reversed-phase HPLC [7–15]. Frequently, GC [16] as well as Micellar electrokinetic capillary chromatography (MECC) are used [17].

Fluconazole given orally and i.v. must be of appropriate chemical and microbiological purity as required by various Pharmacopeia [18,19].These Pharmacopoeia accept the following methods of sterilization: saturated steam, dry heat, chemical sterilization (ethylene oxide), filtration and ionizing radiation.

Radiation sterilization using gamma, beta or E-beam radiation has a number of advantages such as high efficiency, suitability to sterilize thermolabile substances and is less destructive to a number of drugs and materials.

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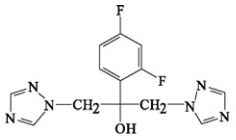

Molecular formula: $C_{13}H_{12}F_2N_6O$
Relative molecular weight: 306.3
Melting point: 138-140°C
Chemical name: α-(2,4-diflurophenyl)-α-(1*H*- triazol-1-methyl)- 1*H*-1,2,4-triazole-1-ethanol
Proprietary names: Biozolene, Diflucan, Elazor, Fluconazole, Flumycon, Fungata, Lavisa, Loitin, Solacap, Triflucan

Fig. 1. Fluconazole—the formula and its basic characteristic according to Moffat et al. [1].

Whether a drug can be sterilized by ionizing radiation depends on its ability to retain its physico-chemical and pharmacological properties. In order to test the resistance of drugs to gamma radiation, doses much higher than the standard sterilizing dose of 25 kGy are used. This allows to detect any changes that the drug might undergo and to observe any products of radiolysis more efficiently than with lower doses.

Analytical control of medicinal products sterilized by radiation should be carried out using the whole range of qualitative and quantitative methods, including hyphenated methods such as TLC–UV, GC–MS and HPLC–MS.

In our recent work [20], we have demonstrated by using DSC and X-ray diffraction that fluconazole irradiated to doses 20–200 kGy showed a lower melting point temperature proportional to the dose received and significant changes in the X-ray spectrum. Observed changes may suggest the existence of radiolysis products and physicochemical changes including polymorphism. In our present work, we wanted to follow up these findings by applying spectroscopic and chromatographic methods. We have used UV, IR and NMR spectroscopy, TLC and HPLC to resolve the question whether fluconazole can be sterilized by ionizing radiation.

2. Experimental

2.1. Material

Fluconazole, Krakowskie Zakłady Farmaceutyczne Pliva SA, LOT:FL02004, content 100.3% (with respect to dry mass).

2.2. Methods

2.2.1. Irradiation with E-beam radiation

Approximately 0.1 g of fluconazole was placed in colourless glass jars of 3 ml volume and closed with a plastic stopper. They were irradiated 20–200 kGy with the help of linear electron accelerator LAE 13/9, the energy of electrons was 9.96 MeV and current intensity 6.2 μA.

2.2.2. Organoleptic analysis

The substance was examined before and after irradiation with respect to their appearance, colour, smell and clarity of the solution obtained by dissolving a particular compound in methanol. All experiments were performed in accordance with Pharmacopoea Polonica, Edition VI, 2002.

2.2.3. Measurement of the degree of dispersion

Scanning electron microscope (SEM 515, Philips) micrographs confirmed that the compound studied had a crystalline structure.

Samples were placed on specimen stubs and fixed with carbon tabs; they were then sputter coated with gold in a sputter coater type SCD 050 Balzers. The stubs were next placed in SEM 515 (Philips) working at 15 kV and magnifications 50×, 250×, 500× and 1000×. Chosen pictures were processed by digital image scanning system (DISS). Particle size was measured manually. About 1000 particles were sampled.

2.2.4. Infrared spectroscopy (IR)

A KBr disc was prepared by mixing 1.00 mg of a substance with 300 mg of KBr and compressing it with Pye Unicam minipress. The spectra were recorded using a Bruker IR spectrometer in the range 500–4000 cm^{-1} with KBr as a blank. The apparatus was calibrated using water and its resolution was 2 cm^{-1}.

2.2.5. Nuclear magnetic resonance (NMR)

Spectra were recorded at 300 K on a Bruker Avance 400 spectrometer, operating at 1H frequency of 400.13 MHz and equipped with Bruker 5 mm QNP probe. Spectra were referenced to the CH_3 signals at 0.0 ppm in TMS. Spectra were acquired by adding either 64 or 128 transients with an acquisition time of 3.95 s, using a 90° pulse of 7.3 μs. Number of data points was 65536. There was an exponential line broadening of 0.30 Hz applied before FT. The resulting spectra were phased manually, baseline corrected, using a quadratic function and integrated manually all using XwinNMR (version 3.5 Bruker).

2.2.6. Ultraviolet spectrophotometry (UV)

The solutions were prepared by dissolving the substance in methanol–water solution (1:4) to obtain concentrations 0.02%, w/v. The solutions were studied using UV–vis Perkin-Elmer Lambda 20 spectrophotometer, in 1 cm cuvettes in the range 200–400 nm, using a solvent as a blank. For the concentrations in the range 0.005–0.045%, the calibration curve was determined expressed by $Y = 21.64x$, characterised by the correlation coefficient of $r = 0.9999$. The precision of the method is described by the variation coefficient $W_Z = 1.36\%$. The limit of detection is $6.1 \times 10^{-4}\%$, while the limit of determination is $1.85 \times 10^{-3}\%$

2.2.7. Thin layer chromatography (TLC)

Plates of dimensions 5.0 cm × 20.0 cm, covered with silica gel Kiesegel 60 F_{254} were used.

The mobile phases listed below were used before [21] with the exception of the last one, which was prepared by the authors:

- toluene–isopropanol–25% ammonia (8.4:3.5:0.1, v/v/v);
- chloroform–methanol–water (13.3:1.5:0.2, v/v/v);
- cyclopean–ethylene chloride–methanol–100% acetic acid (2.2:11.3:1.5:0.1, v/v/v);
- 1-butanol–water–100% acetic acid (11.6:3.3:0.1, v/v/v);
- chloroform–aceton–methanol–25% ammonia (4:4:1:0.1, v/v/v/v).

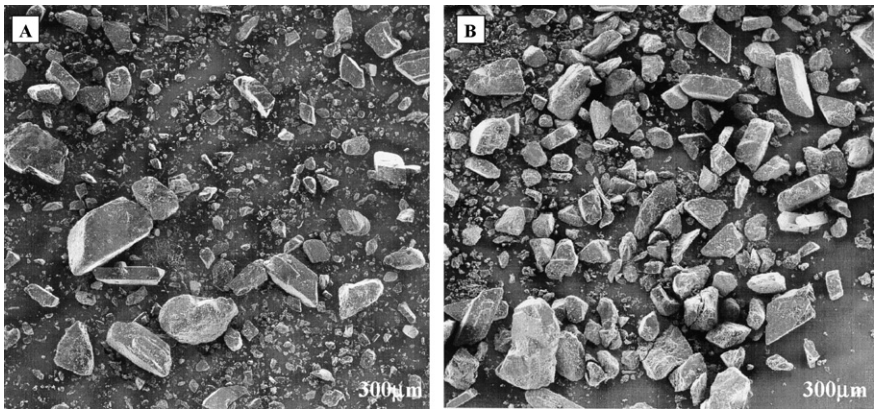

Fig. 2. SEM picture of fluconazole before (A) and after (B) E-beam radiation (200 kGy).

The 25 μl of 1% solution of fluconazole, i.e. 0.25 mg of substance was placed on each plate. The spots were set with a quartz lamp working at $\lambda = 254$ nm.

2.2.8. High-performance liquid chromatography (HPLC)

Purosphere STAR RP-18 capillary column, 55 mm × 4 mm dimension and 3 micrometer diameter was used.

The mobile phase consisted of: $NaH_2PO_4 \times H_2O$–methanol–acetonitrile (82.7:7.1:10.2, v/v/v). The rate of flow was 1.5 ml/min and the UV detector was set at 254 nm. The separation was conducted at room temperature on the Merck–Hitachi instrument.

The range of concentrations for method calibration and for analytical studies was 62.5–1000 mg/l (respectively, 62.5; 125.0; 250.0; 500.0; 1000.0 mg/l). The precision of the HPLC method was characterised by relative standard deviation (R.S.D) of 2.54–2.92. The quantitation limit is 3.12 mg/l. The limit of detection, defined with a signal-to-noise ratio better than 3, was 0.47 mg/l. Statistical analysis was performed using the licensed program Excel, V 2000.

3. Results and discussion

Organoleptic analysis of fluconazole showed that even low irradiation doses gave rise to significant colour change of samples. The non-irradiated sample of fluconazole was a white, crystalline powder. After a 20 kGy dose, the colour changed to cream and with doses of 50–100 kGy to salmon pink and finally orange at 200 kGy.

The SEM picture did not reveal any changes in the crystalline structure of fluconazole or the size of the crystals (Fig. 2 and Table 1). Small differences in the percent content of some crystals are most likely due to the error of the method. The SEM results are identical to the ones published in our earlier work [20]. The changes in the XRD spectrum of fluconazole observed in ref. [20] suggest either formation of products of radiolysis or some other physicochemical changes. For that reason, we have decided to use IR spectroscopy in the present work; however, no significant changes were observed between the irradiated sample of fluconazole and the original (Fig. 3) with the exception of a small decrease in the intensity of absorbance in the whole spectrum. The lack of any changes in the IR spectrum suggest either that new products are formed in trace amounts (what was observed for derivatives of imidazole [22–24] or that they are structurally almost identical to the original compound so their spectra overlap with the original non-irradiated substance.

The ^{1}H-NMR results of irradiated and non-irradiated samples of fluconazole did not show any relevant differences either (Fig. 4). The CH_2 protons of nonirradiated fluconazole resonated between 4.65 and 4.9 ppm, the CH protons on the triazole ring resonated at 7.8 and 8.3 ppm, respectively, and the benzene protons at 6.8, 6.95 and 7.3. The same values of chemical shift were observed for the irradiated fluconazole, supporting the finding of the IR experiments.

Table 1
Particle size distribution (%) determined from SEM micrographs

Particle size (μm)	Content (%)	
	0 kGy	200 kGy
0–50	58.5	63.3
50–100	20.1	18.5
100–150	11.5	9.5
150–200	4.5	4.1
200–250	3.0	2.4
250–300	1.4	1.2
300–350	0.6	0.7
350–400	0.4	0.3

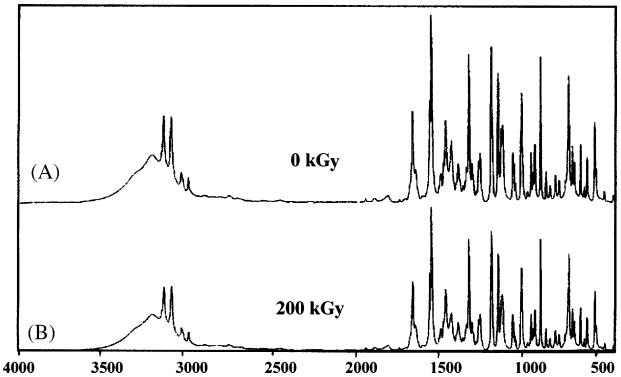

Fig. 3. Infrared spectrum of fluconazole before (A) and after (B) E-beam radiation.

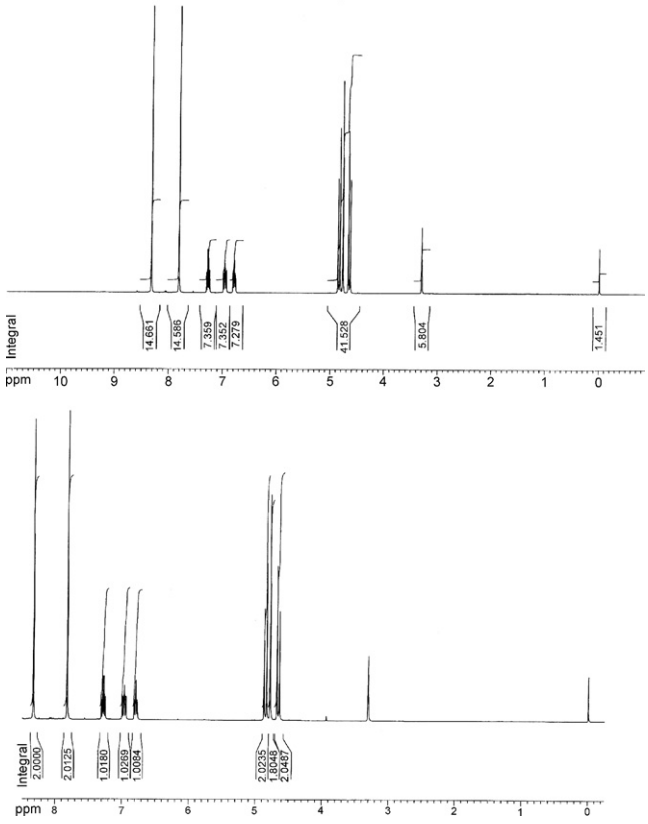

Fig. 4. ^{1}H-NMR spectrum of fluconazole before and after E-beam radiation.

The UV spectra of water–methanol solutions of fluconazole showed an increase in absorbance at λ_{max} with the increase in radiation dose. For the dose of 200 kGy this increase was 37%. Also, an additional absorption band appeared between 280 and 310 nm (Fig. 5), suggesting the presence of some radiolysis products with chromophores absorbing in the above range.

The TLC analysis conducted in four mobile phases, recommended by ref. [21] showed that fluconazole did not contain any impurities or decomposition products before or after irradiation.

A different mobile phase consisting of chloroform–acetone–methanol–25% ammonia (4:4:1:0.1) was tried in the search for

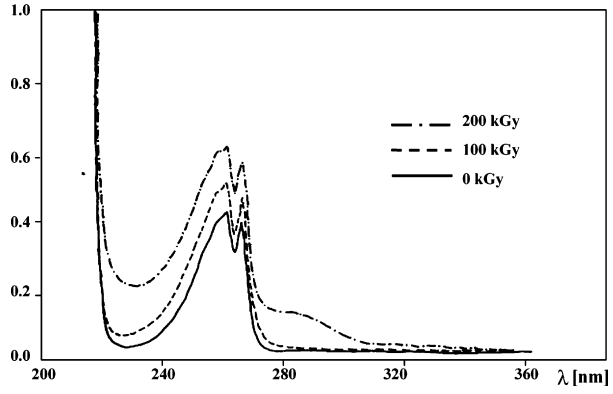

Fig. 5. UV spectrum of 0.02%, w/v solution of fluconazole in methanol–water (1:4).

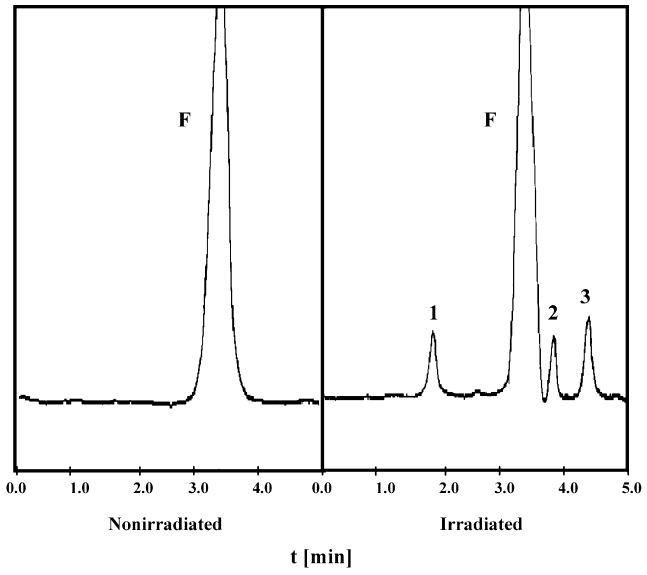

Fig. 6. HPLC trace of fluconazole irradiated to 200 kGy. (F) Fluconazole; (1,2,3) products of radiolysis.

decomposition products. One product of radiolysis was found with R_f of 0.80 for a sample irradiated to 100 kGy and two with R_f of 0.77 and 0.82 for a sample irradiated to 200 kGy (Table 3). Samples of fluconazole that received doses smaller than 100 kGy did not show any radiolysis products.

The HPLC results not only confirmed the ones obtained by TLC, but also showed higher sensitivity by identifying more products of radiolysis. For example, a sample irradiated to 100 kGy showed two additional signals with retention times t_R = 1.84 and 4.26 min and one that received 200 kGy showed three additional peaks with t_R = 1.84, 3.66 and 4.26 min (Fig. 6 and Table 2). The HPLC chromatograms for fluconazole irradiated to lower doses did not indicate the existence of any radiolysis products.

The UV spectra of products of radiolysis, separated by the HPLC, showed λ_{max} close to the initial compound (Fig. 7 and Table 2) and could therefore interfere with the interpretation of the results by the UV method. For this reason, UV spectrophotometry is unsuitable for quantitative determination of fluconazole giving values higher than expected. The HPLC method seems the most suitable for the purpose of determining decomposition products of radiolysis of fluconazole (Table 3). This sensitive and accurate technique found that the amount of fluconazole decreases with the increase of irradiation dose

Table 2
The HPLC and TLC results of fluconazole before and after irradiation

Dosis (kGy)	Products of radiolysis		Retention time, t_R (min)	Content (%)	
	TLC	HPLC		UV	HPLC
0	0	0	3.30	100.0	100.00
20	0	0	3.30	104.9	99.52
50	0	0	3.30	107.3	98.12
100	1	2	1.84; 3.30; 4.26	104.1	95.69
200	2	3	1.84; 3.30; 3.66; 4.26	137.9	92.88

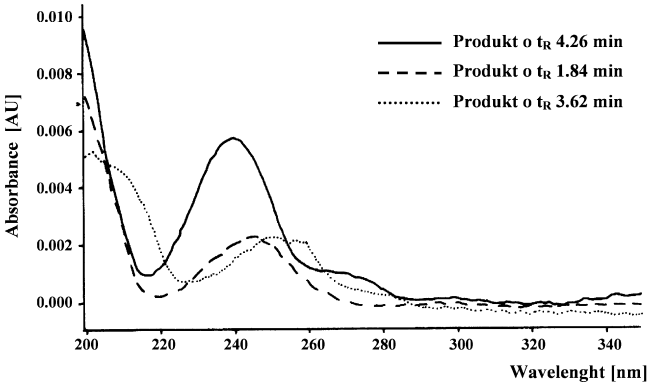

Fig. 7. Ultraviolet spectrum of radiolysis products of fluconazole.

Table 3
The UV and HPLC parameters of products of radiolysis of fluconazole after irradiation 200 kGy

Compound	TLC (R_f)	HPLC, t_R (min)	λ_{max} (nm)
Flukonazole	0.53	3.30	251
Product I	0.77	1.84	250; 258
Product II	–	3.62	244
Product III	0.82	4.26	238; 270

Table 4
Fluconazole irradiated to 200 kGy: comparison of results obtained from various analytical methods

Method	Results
Organoleptic analysis	Change of colour
SEM	No change
IR	No change
UV	Increase of absorbance at λ_{max} and an additional absorption band in the range 280–310 nm
NMR	No change
TLC	Two products of radiolysis
HPLC	Three products of radiolysis decrease of content

and the appearance of decomposition products. A linear dependence with a very good correlation coefficient, $r = 0.9926$ was obtained between the doses (20–200 kGy) and the content of fluconazole.

Having reviewed the last 30 years of research on fluconazole, we did not find any information on its resistance to ionising radiation or on sterilisation of fluconazole using ionising radiation. For this reason, we cannot compare our results, gathered in Table 4 with the results of other authors. However, we hope to understand the mechanism of radiolysis of fluconazole by isolating and identifying decomposition products by the HPLC method.

4. Conclusions

The physico-chemical properties of fluconazole change during sterilisation with ionizing radiation (E-beam radiation). Even the smallest dose of 20 kGy alters the colour from white to cream and with the further increase of the dose, the colour deepens from salmon pink at 50 kGy to orange at 200 kGy. This change in colour is caused by the formation of products of radiolysis with a subsequent lowering of the content of fluconazole, the active ingredient. Therefore, we conclude that fluconazole is not radiochemically stable and should not be sterilized by ionizing radiation.

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