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Study of nonvolatile degradation compounds produced by radiosterilization of cefotaxime

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Abstract

The effects of radiosterilization on the purity profile of cefotaxime were evaluated by a liquid chromatography-diode array method. Numerous new radiolytic compounds were detected in very small amount. They were quantified and it appeared that none was present above the level of 0.1% of the main compound and the total amount was only of 0.72%. Despite the low quantities present, some radiolytic compounds had UV spectra which could justify the apparition of a yellow coloration detected after irradiation. Others had UV spectra similar to that of cefotaxime, suggesting similarity in the molecular structures. Finally, some mechanisms of formation were proposed for four radiolytic compounds which were identified by mass spectrometry in a former study.

Keywords: Radiosterilization; Cefotaxime; Color modification; HPLC; Lactam; Radiolytic compounds

1. Introduction

The use of gamma irradiation to sterilize medical devices such as catheters, sutures, syringes, etc. was introduced 30 years ago. This technique is now widely used in many countries. On the contrary, radiosterilization of pharmaceutical compounds has developed less quickly and the number of irradiated drugs present in the market remains low. Most of them are parenteral drugs, veterinary compounds and ophthalmic ointments.

However, pharmaceutical industries are more and more interested in this technique. The main advantage of radiosterilization arises from the high penetrating power of the gamma-rays allowing a terminal sterilization which permits to avoid any risk of further contamination. Another positive point is the isothermal character of the process (Jacobs, 1985; Tilquin, 1991). It is thus perfectly suitable for heat-sensitive drugs like cephalosporins for instance. Furthermore, radiosterilization should become more and more important, especially to sterilize new drug delivery systems and pharmaceuticals from biotechnology, impossible to sterilize by other ways (Reid, 1995).

On the other hand, the irradiation treatment can produce new potentially toxic radiolytic products and consequently modify the purity profile of irradiated drugs.

The feasibility of radiosterilization of many pharmaceutical compounds has been investigated and several review papers have been published (Gopal, 1978; Jacobs and Wills, 1988; Schüttler and Bögl, 1992, 1993, 1994; Boëss and Bögl, 1996). Most of these studies have essentially been descriptive but few radiolytic compounds have been identified.

The recent improvements of analytical techniques such as chromatography coupled to mass spectrometry allows to envisage the identification of these radiolytic products even present in trace amounts and would be very useful to understand the radiolytic mechanisms of their formation.

The present work is concerned with the effects of radiosterilization on cefotaxime sodium salt. Its

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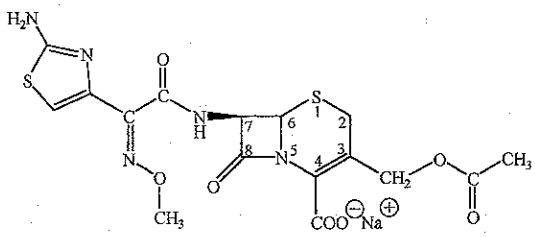


Fig. 1. Molecular structure of cefotaxime sodium salt.

structure is presented in Fig. 1. Owing to its high activity against both gram-positive and gram-negative bacteria and its resistance to β-lactamases, cefotaxime is a very useful antibiotic that belongs to the cephalosporin group. Because of its poor stability in solution, it is commercialized as a sterile powder needed to be dissolved just before administration. Up to now, this thermosensitive compound has been sterilized by the expensive process of microfiltration consisting of filtration, lyophilization and filling steps under aseptic conditions. So, for this kind of compounds, gamma irradiation is a very interesting alternative method of sterilization.

Although radiosterilization does not modify its activity (Zegota et al., 1995), it produces new radiolytic products responsible for a modification of color and odor, due to volatile and nonvolatile compounds formation, respectively. These modifications could lead to the nonconformity of the irradiated drug with the different Pharmacopoeias.

The volatile compounds produced by irradiation of cefotaxime and other cephalosporins were previously studied (Barbarin et al., 1996) and their origin was also investigated (Barbarin et al., 1999).

The aim of this present work is to study the nonvolatile radiolytic compounds. The loss of potency of cefotaxime was first evaluated by HPLC. The new radiolytic compounds were then analyzed and quantified. Finally, mechanisms of formation of some compounds, identified by liquid chromatography-electrospray mass spectrometry (Barbarin et al., submitted), were proposed.

2. Experimental

2.1. Materials

Cefotaxime sodium salt (Claforan®) was kindly provided by Hoechst. A working standard of cefotaxime, also provided by Hoechst, was used for quantitative experiments (batch D631 WST-7, purity = 98.4%).

Methanol of super-gradient grade was supplied by Lab-Scan (Dublin, Ireland), ammonium acetate by Merck (Darmstadt, Germany) and water was deionized in the laboratory by a Milli-Q system (Millipore-Waters, Milford, MA, USA).

2.2. Irradiation

The irradiation was performed under an atmosphere of air and at ambient temperature in the panoramic ^{60}Co chamber in Louvain-la-Neuve (Catholic University of Louvain, Belgium). The absorbed dose of 25 kGy was measured using the Fricke dosimeter. The dose rate was 0.17 Gy/s.

2.3. HPLC instruments and chromatographic conditions

The HPLC system consisted of a Merck-Hitachi L6200 gradient pump equipped with a Merck-Hitachi AS2000 autosampler and a L4500 diode array detector. The Merck-Hitachi D-7000 HSM HPLC system manager software was used to control the pump and for data acquisition.

The chromatographic separation was performed on a 250 × 4 mm Lichrospher® RP Select B column, 5 µm particle size (Merck, Darmstadt, Germany). The mobile phase consisted of methanol and a 10 mM ammonium acetate aqueous solution. The pH was adjusted to 4 with 0.1 M HCl. The following gradient conditions were used: $t = 0'$: buffer/methanol (95 : 5); $t = 5'$: buffer/methanol (95 : 5); $t = 60'$: buffer/methanol (70 : 30). The absorbance was measured between 200 and 400 nm with a spectral resolution of 4 nm. The monitoring and the quantification wavelength was set at 235 nm corresponding to the absorption maximum of cefotaxime. A flow rate of 1 ml/min and an injection volume of 20 µl were used. Analyses were performed at room temperature.

The loss of potency after irradiation was determined with a 10^{-3} M aqueous solution of cefotaxime. The search for new radiolytic compounds were made by injecting a 10^{-2} M aqueous solution of cefotaxime.

3. Results and discussion

3.1. Loss of potency

To evaluate the stability of cefotaxime to gamma radiation, its peak area was compared before and after irradiation.

The mean of the peak area value of four different analysis coming from four different solutions was made. The results are presented in Table 1.

These results showed no significant difference between the two means and suggested a very high resistance of cefotaxime to gamma irradiation. Therefore, the

Table 1
Comparison between the peak area of nonirradiated and irradiated cefotaxime

	Peak area of nonirradiated cefotaxime	Peak area of irradiated cefotaxime
Mean	2 146 298 ($n = 4$)	2 158 644 ($n = 4$)
Standard deviation	40 130	28 312
RSD (%)	1.87	1.31

possible degradation compounds were produced in so low amount that no decrease of the drug substance peak area was observed.

3.2. Detection of radiolytic compounds

To detect possible degradation compounds, a more concentrated solution of cefotaxime was analyzed (10^{-2} M). The chromatograms of nonirradiated and irradiated samples are presented in Fig. 2.

3.2.1. Study of the chromatograms

Several impurities present before irradiation were detected in the nonirradiated sample (Fig. 2a). The most important ones were designed by letters (from a to k) but others were also detected in very small amount. Among them, two were already identified by coelution experiments (Crucq, 1995) as deacetylcefotaxime (peak b) and deacetoxycefotaxime (peak f). These are classical impurities of cefotaxime and are listed in the monography of the European Pharmacopoeia.

Fig. 2b presents the chromatogram of an irradiated sample. The results showed that:

- 15 extra peaks appeared with the irradiation.
- Moreover, the beginning of the chromatogram showed the presence of other small peaks, not separated and corresponding probably to polar compounds.
- Peak N°s. 4, 5 and 11 were not pure and contained at least two different products each.
- The radiolytic compounds were produced in very small amount: their peaks were much smaller than those of the impurities already present before irradiation.
- On the contrary of what is postulated by Nordhauser and Olson (1998), the radiolytic compounds seemed to be unique to the irradiation, suggesting unique radiolytic mechanisms of formation.

3.2.2. Study of the UV spectra

The comparison between the UV spectra of some of the radiolytic compounds (presented in Fig. 3) and the UV spectrum of cefotaxime (presented by Zegota et al., 1995) shows that most of them (UV spectra of peaks nos. 1, 6, 7, and 13) looked very similar to the one of the reference compound. Moreover, they had the same absorption maximum at 256 nm which is characteristic of an Δ3-cephem chromophore and thus an unaltered cephem ring (Hou and Poole, 1971; Van Krimpen et al., 1987). These results suggested that these compounds had a molecular structure similar to that of cefotaxime.

Some other radiolytic compounds (peak nos. 11, 12, and 14) presented an absorption maximum in the zone of 320–380 nm and could therefore be responsible for the apparition of a yellow color with the irradiation.

The other compounds were present in too small amount (peak nos. 3, 4, 5, and 10) or were coeluted (peak nos. 8 and 15). Thus, no valuable UV spectra were obtained for them.

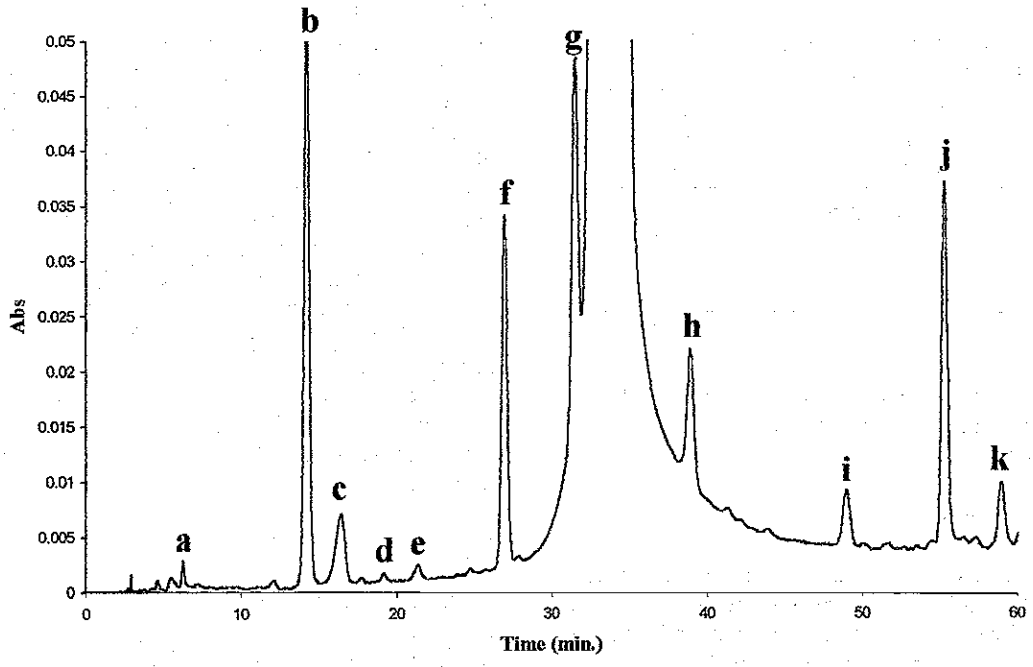
3.3. Dosage of the related compounds

The percentage of the main impurities, present before irradiation, and that of the radiolytic compounds were determined. The compounds being unknown, it was supposed that their molar absorptivities were identical to that of cefotaxime. Thus, calculations were performed using the response factor of cefotaxime, as recommended by the ICH guidelines (ICH Q3A, 1995; ICH Q3B, 1996). This response factor was determined owing to a working standard of known purity. As shown in Fig. 3, this is true for most of the compounds. However, some others (peak nos. 11 and 12, for example) had different UV spectra and therefore probably different molar absorptivities. The percentages of these compounds must thus be taken carefully.

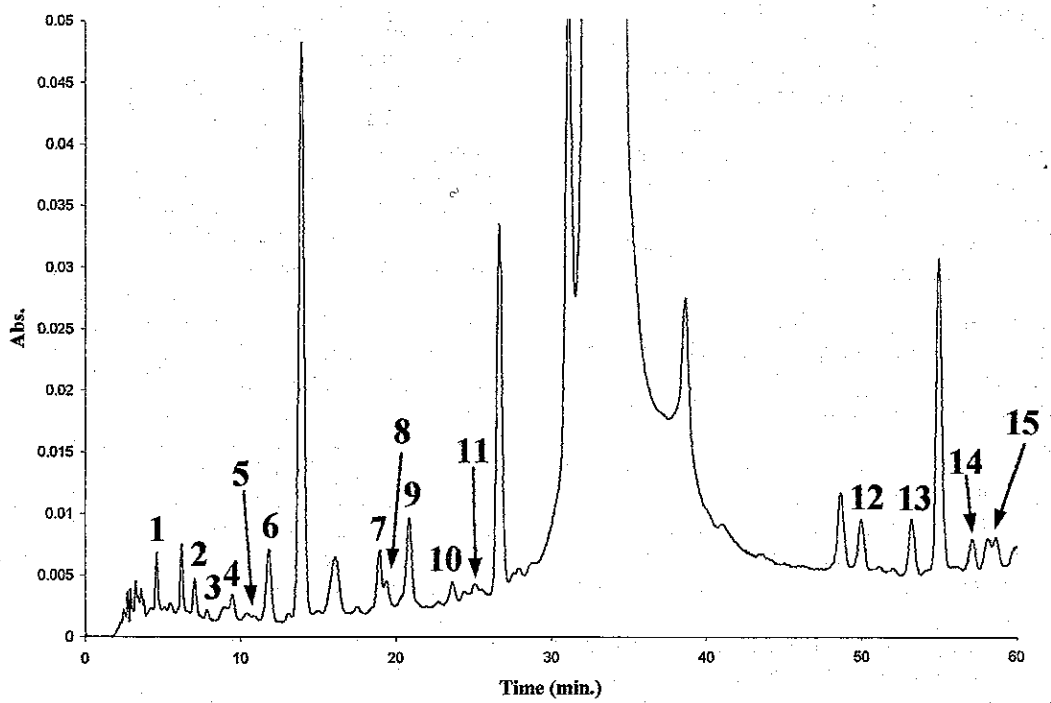
The results are presented in Tables 2 and 3 and showed the very small amount of radiolytic compounds produced. Every detected radiolytic peak had a level below 0.1% of the main compound and the total amount was only of 0.72%. The first line of Table 2, called "peaks eluted before 5", included several compounds, badly separated and therefore quantified together. For comparison, the total quantity of impurities present before irradiation was 2.57% with an amount of deacetylcefotaxime and deacetoxycefotaxime of 0.74% and 0.54%, respectively. The amount of radiolytic compounds was thus much lower than that of the existing impurities.

3.4. Mechanisms involved

In order to identify the radiolytic compounds, a liquid chromatography-electrospray mass spectrometry method was developed and is described elsewhere (Barbarin et al., submitted). This study allowed to propose a structure for three new radiolytic compounds and to confirm the presence of anticefotaxime, already



(a) non irradiated cefotaxime



(b) irradiated cefotaxime

Fig. 2. : Chromatogram of (a) nonirradiated and (b) irradiated cefotaxime. λ of detection = 235 nm.

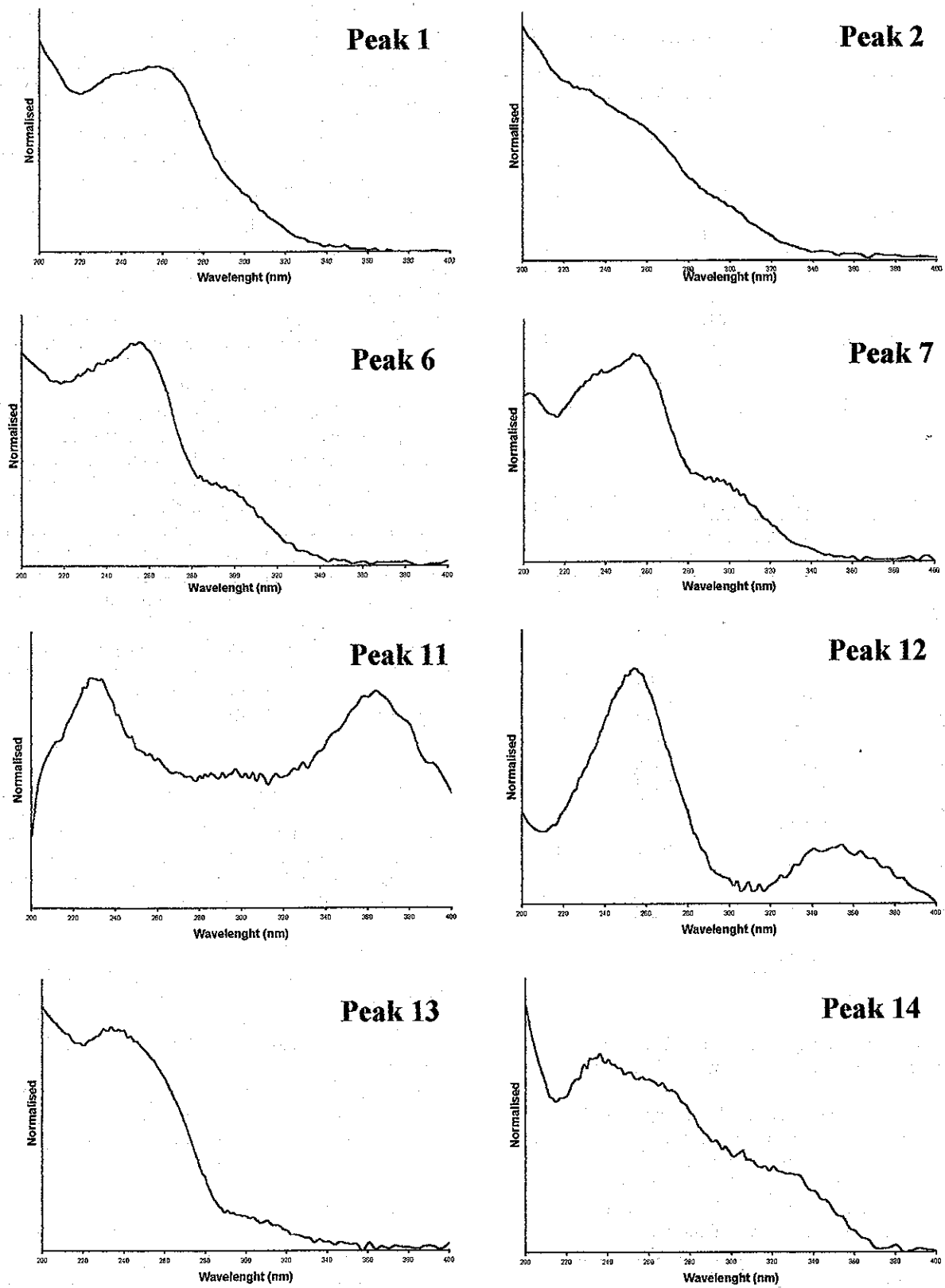


Fig. 3. UV spectra of some radiolytic compounds.

Table 2
Percentage of impurities present in a nonirradiated sample of cefotaxime

Peak	Mean percentage ($n = 3$)	Standard deviation	RSD (%)
a	0.02 %	0.0005	2.5
b	0.74 %	0.03	4.1
c	0.18 %	0.004	2.2
d	0.02 %	0.0003	1.5
e	0.02 %	0.003	1.5
f	0.45 %	0.006	1.3
g	0.32 %	0.02	6.3
h	0.21 %	0.009	4.3
i	0.1 %	0.004	4
j	0.41 %	0.01	2.4
k	0.1 %	0.01	10
Sum	2.57 %		

Table 3
Percentage of radiolytic compounds produced by irradiation

Peak no.	Mean percentage ($n = 3$)	Standard deviation	RSD (%)
Peaks eluted before 5'	0.14 %	0.008	5.7
1	0.05 %	0.001	2
2	0.03 %	0.0007	2.3
3	0.003 %	0.0004	13.3
4	0.04 %	0.0005	1.3
5	0.005 %	0.001	20
6	0.09 %	0.002	2.2
7	0.07 %	0.0008	1.1
8	0.05 %	0.003	6
9	0.02 %	0.002	10
10	0.02 %	0.002	10
11	0.03 %	0.002	6.6
12	0.04 %	0.002	5
13	0.08 %	0.001	1.25
14	0.05 %	0.002	4
Sum	0.72 %		

described previously (Crucq and Tilquin, 1996 a,b). The structures of these four compounds are presented in Fig. 4.

It can be noted that the chromatograms obtained by MS were quite different from those presented in Fig. 2. Indeed, some compounds had a high molar absorptivity and gave an intense signal in UV but were not detected by mass spectrometry. It is the case of the polar compounds eluted at the beginning of the analysis, for example. On the contrary, some peaks detected by MS were not detectable by UV. Therefore, it was difficult to establish a correlation between the results obtained by UV and MS and thus to attribute a peak number to the proposed structures. Only peak no. 13 could be attributed to anticefotaxime. Consequently, it was also difficult to know if the identified compounds presented in Fig. 4 were those responsible for the color modification (peak nos. 11, 12, and 14). However, the apparition of a yellow coloration being due to formation of compounds with high delocalized π electrons, it would be surprising that the identified structures were responsible for color modification.

The results obtained previously by mass spectrometry about the volatile and nonvolatile radiolytic compounds of cefotaxime (Barbarin et al., 1996, 1999), together with the results presented in this study allowed us to understand better the mechanisms involved in their formation.

3.4.1. Anticefotaxime (compound I)

Anticefotaxime was already identified previously (Crucq and Tilquin, 1996 a, b) and is also the major degradation compound after UV irradiation. (Lerner et al., 1988). It is the diastereoisomer "E" of cefotaxime coming from a *cis–trans* isomerization of the oxime function. The presence of this compound supported the hypothesis of a very important sensitivity of this oxime function to the gamma rays, as already suggested by the presence of volatile oxime compounds detected previously (Barbarin et al., 1996, 1999).

Furthermore, its presence showed the potentiality for gamma irradiation to produce stereoisomers, as already shown by the study performed by mass spectrometry (Barbarin et al., submitted).

3.4.2. Compound II

Compound II came from an interaction between the oxime function and the thiazol ring of cefotaxime, leading to the formation of a new ring, very well stabilized by resonance. The formation of this compound is another evidence of the very high sensitivity of the oxime function to gamma irradiation.

3.4.3. Compound III

An hypothetical mechanism of compound III formation is proposed in Fig. 5. The loss of a $H^{\cdot}$ radical from the position 2 of the cephem nucleus followed by an oxidation could lead to the formation of a ketone function.

The presence of this radiolytic compound could explain, by subsequent rupture of the cephem ring, the formation of carbon oxide sulfide (COS) detected after irradiation of cefotaxime (Barbarin et al., 1996, 1999).

3.4.4. Compound IV

Structure IV corresponds to *N*-formylcefotaxime which is a well-known impurity of cefotaxime, listed in the European Pharmacopeia (Eur. Pharm., 2000) and which was detected by LC-MS in the nonirradiated

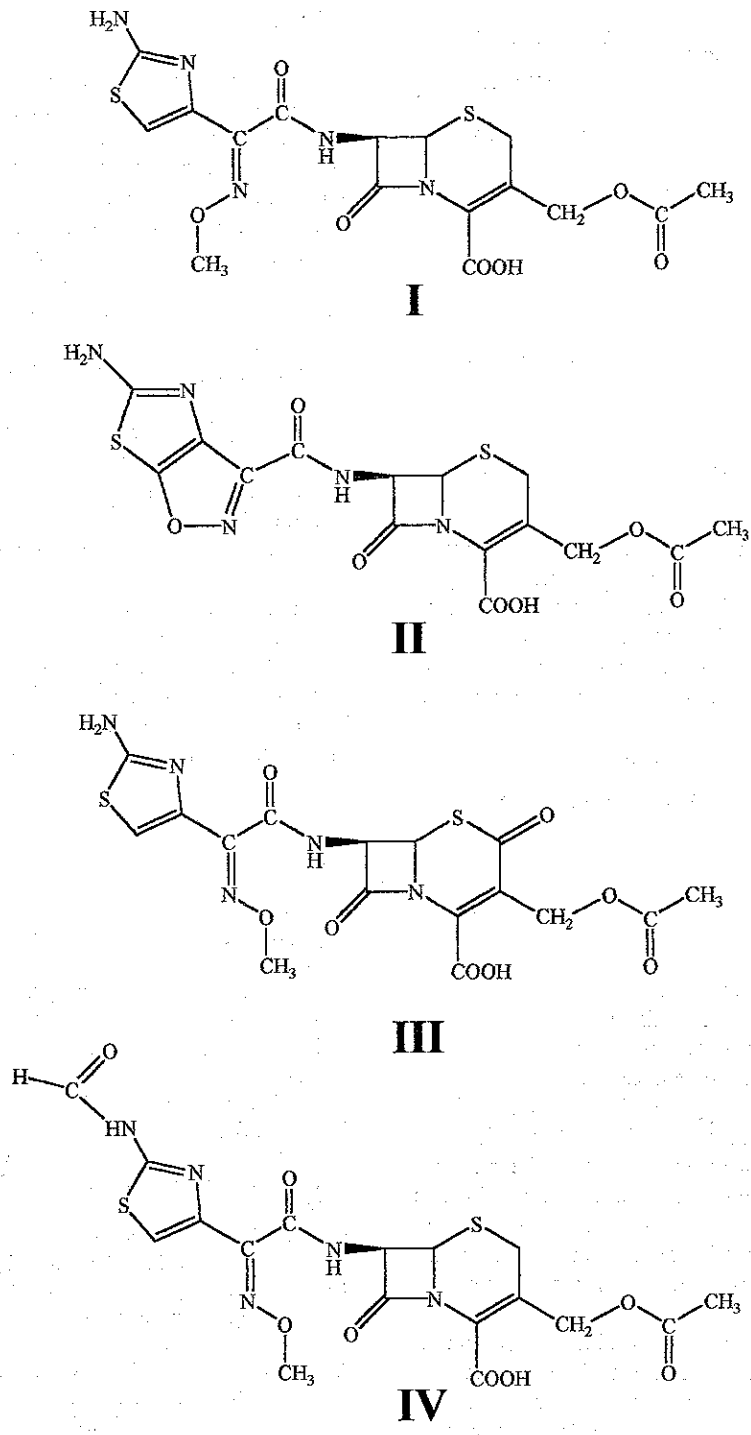


Fig. 4. Molecular structures of identified radiolytic compounds of cefotaxime.

sample (Barbarin et al., submitted). These results also showed the apparition of a diastereoisomer of *N*-formylcefotaxime with the irradiation. Unfortunately, the structure in space of this radiolytic compound remains unknown.

Two hypotheses could be advanced to explain the origin of it:

- It could directly come from the stereoisomerization of *N*-formylcefotaxime.

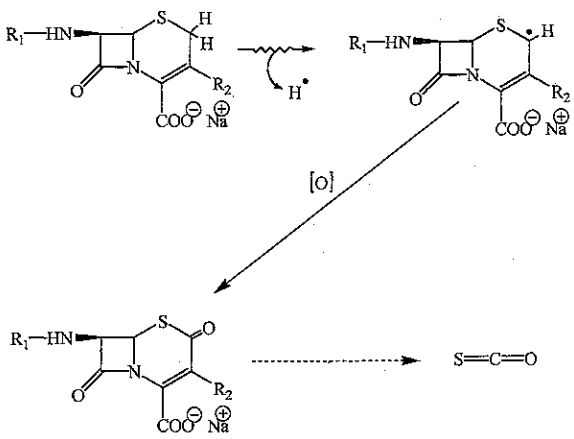


Fig. 5. Proposed mechanism of compound III formation.

- It could come from a reaction between an intact molecule of cefotaxime and a formyl radical coming from another molecule of cefotaxime as described below. The presence of this radical was demonstrated previously (Barbarin et al., 1999) and was also involved in the appearance of the volatile ester compounds:

$$\text{Cefotaxime} \rightarrow (\text{HCO})^{\bullet}$$

$$(\text{HCO})^{\bullet} + \text{Cefotaxime} \rightarrow N\text{–formylcefotaxime}$$

4. Consequences of radiosterilization on the quality of cefotaxime

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use adopted two guidelines related to impurities in new drug substances and in new drug products (ICH guidelines Q3A and Q3B, 1995, 1996).

These guidelines specify that impurities below the apparent level of 0.1% (calculated using the response factor of the drug substance) should not be identified. It means that, in the case of cefotaxime, identification of the radiolytic compounds is not required. However, it is very useful to understand better the radiolytic mechanisms involved.

Despite the small amount of radiolytic compounds formed, the irradiation of cefotaxime caused the apparition of a yellow coloration which was not conform with the requirements of the Pharmacopoeias. These results could be explained by the high molar extinction coefficient of the radiolytic compounds and could explain why some compounds were better detected by UV than by mass spectrometry. Consequently, the UV–VIS detection is still very useful and should not be neglected in the studies of the radiolysis of the pharmaceuticals.

5. Conclusions

Radiosterilization of cefotaxime led to the formation of unique nonvolatile compounds in very small amount. None of these compounds had a level higher than 0.1% of cefotaxime which corresponds to the level above which the identification is required by the ICH guidelines. Moreover, the total amount was much lower than the that of the impurities present before irradiation.

The similar UV spectra of the radiolytic compounds and cefotaxime suggested close molecular structures. Moreover, the UV spectra of some of them could explain the apparition of a yellow coloration.

Although not required, the previous identification of four radiolytic compounds allowed to propose some mechanisms involved in their formation and to connect the results with those obtained with the study of volatile compounds.

Finally, it appears that LC-MS and LC-DAD are two complementary methods for the study of radiosterilization of pharmaceuticals. If the first one was essential to identify the radiolytic compounds and therefore to understand better the mechanisms involved in their formation, the use of a DAD detector allowed to detect and to quantify very low amount of these compounds and to locate those responsible for the modification of coloration.

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