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# THE RADIOLYTIC STUDIES OF CEFTRIAXONE IN THE SOLID STATE

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**Abstract:** The possibility of applying radiation sterilization to ceftriaxone disodium in the solid state was investigated. The lack of significant changes in the assay of ceftriaxone disodium irradiated with a dose of 25 kGy, required to attain sterility, was confirmed. The antibacterial activity of ceftriaxone disodium irradiated with a dose of 25 kGy, was unaltered for Gram-positive bacteria except of *Staphylococcus aureus* and changed for Gram-negative strains with except of *Klebsiella pneumoniae*. *Proteus vulgaris* showed the greatest sensitivity to CTD even after the application of radiation sterilization of 400 kGy.

**Keywords:** ceftriaxone disodium, radiation sterilization, stability

Ceftriaxone disodium (CTD) (Fig. 1) is a third-generation semisynthetic cephalosporin with a long half-life which has resulted in a recommended once daily administration schedule. It is administered intravenously or intramuscularly and has a broad spectrum of activity against Gram-positive and Gram-negative aerobic and some anaerobic bacteria. CTD is effective in complicated and uncomplicated urinary tract infections, lower respiratory tract infections, skin, soft tissue, bone and joint infections, septicemia and pediatric meningitis. In most of above mentioned infections once-daily administration appears efficacious.

CTD similarly to other cephalosporins have surprisingly few serious side effects, but the most of them are caused by the generation of degradation products. For example, fatal immune hemolytic anemia could be stimulated by a degradation product of ceftriaxone (1) so it is important to estimate the stability of cephems. The stability and mechanism of CTD degradation in aqueous solution (2) and in solid state (3) were investigated in previous studies. Other studies have confirmed that cephalosporins are susceptible to degradation in aqueous solutions (4-11) and in the solid state (3, 12-18). Our investigations focused on determining the influence of radiation on CTD at doses of 25 (recommended for sterility) (19), 50, 100, 200 and 400 kGy.

## EXPERIMENTAL

### Standards and reagents

Biotrakson is a sterile powder containing ceftriaxone disodium, for preparation of injections (Bioton Sp. z o.o. Warszawa, Poland). All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical grade. High quality pure water was prepared by using a Millipore Exil SA 67120 purification system (Millipore, Molsheim, France).

### Irradiation

Twenty-five mg samples of CTD were placed in 3 mL colorless glass vials that were closed with plastic stoppers. The samples in the vials were exposed to β irradiation in a linear electron accelerator LAE 13/9 (9.96 MeV electron beam and 6.2 μA current intensity) until they absorbed doses of 25, 50, 100, 200 and 400 kGy.

### Kinetic analysis

For the kinetic study, the Dionex Ultimate 3000 analytical system consisted of a quaternary pump, an autosampler, a column oven and a diode array detector. As the stationary phase a Kinetex with 5 μm core-shell particles, C18, 100A, 100 × 2.1 mm column was used. The mobile phase was com-

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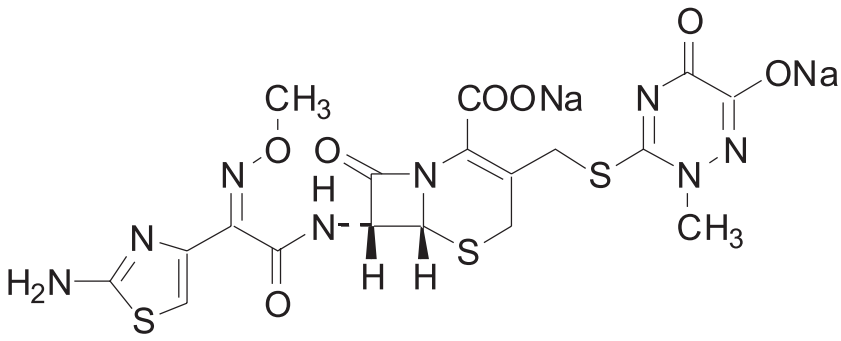

Figure 1. Chemical structure of ceftriaxone disodium

posed of acetonitrile – ammonium acetate (5 : 95, v/v). The flow rate of the mobile phase was 1.0 mL/min and the injection volume was 10 µL. The wavelength of the DAD detector was set at 254 nm. Separation was performed at 30°C.

The stability tests were performed according to the International Conference on Harmonization Guidelines (20).

**Method validation**

HPLC method was validated according to International Conference on Harmonization Guidelines. The method was validated for parameters such as specificity, linearity, precision, accuracy and robustness.

***Selectivity***

The selectivity was examined for non-degraded and degraded samples in aqueous solutions in conditions of acid, base and neutral hydrolysis and in the solid state (thermal and radiolytic degradation).

***Linearity***

Linearity was evaluated in the concentration range 42.4–508.8 mg/L (10-120% of the nominal concentration of CTD during degradation studies). The samples of each solution were injected three times and each series comprised 7 experimental points.

***Accuracy, as recovery test***

The accuracy of the method was determined by recovering CTD from the placebo. The recovery test was performed at three levels 80, 100 and 120% of the nominal concentration of CTD during degradation studies. Three samples were prepared for each recovery level. The solutions were analyzed and the percentages of recoveries were calculated.

***Precision***

Precision of the assay was determined in relation to repeatability (intra-day) and intermediate precision (inter-day). In order to evaluate the repeatability of the methods, six samples were determined during the same day for three concentrations of CTD. Intermediate precision was studied comparing the assays performed on two different days.

***Limits of detection (LOD) and quantification (LOQ)***

The LOD and LOQ parameters were determined from the regression equation of CTD: LOD = 3.3 $S_y/a$, LOQ = 10 $S_y/a$; where $S_y$ is a standard error and $a$ is the slope of the corresponding calibration curve.

***Robustness***

The robustness of the procedure was evaluated after changing the following parameters: the composition of the mobile phase; content of acetonitrile 5 ± 1 [%], the mobile phase flow rate 1.0 ± 0.2 [mL/min]; wavelength of detection 254 ± 5 [nm] and temperature 30 ± 2 [°C]. For each parameter change, its influence on the retention time, resolution, area and asymmetry of peak was evaluated. No significant changes in resolution and shapes of peak, areas of peak and retention time were observed when above parameters were modified. Modifications of the composition of the mobile phase: organic-to-inorganic component ratio resulted in the essential changes of retention time and resolution in determination of CTD.

**Microbiological analysis**

Indicator microorganisms (*Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 14028, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATTC 33420 and *Klebsiella pneumoniae* ATCC 31488) were cultured in soy-casein broth with yeast extract for microorganisms with increased nutritional requirements. *Clostridium butyricum* ATCC 860 and *Clostridium pasterianum* ATCC 6013 were grown in Reinforced Clostridial Medium (RCM, Oxoid, UK). Bacteria were cultured under aerobic or anaerobic conditions (37°C, 24 h) (depending on stains). Minimum inhibitory concentration (MIC) endpoints were determined by broth microdilution according to CLSI guidelines (21). The concentrations of irradiated CTD were 0.02–256 μg/mL. Then, irradiated CTD of decreasing concentrations were added to each of test tubes. Next, test tubes were inoculated with the same amount of cells suspension. After 16-18 h of incubation at 37°C, the growth of strains was checked *via* turbidity increase observation. In test tubes containing less than MIC of examined drugs the turbidity increase was observed (the cells have grown). The minimal concentration of drugs that inhibited strain growth was defined as MIC.

## RESULTS AND DISCUSSION

The HPLC method for determination of CTD was found selective in the presence of degradation product as shown in Figuress. 2 and 3. In the chro-

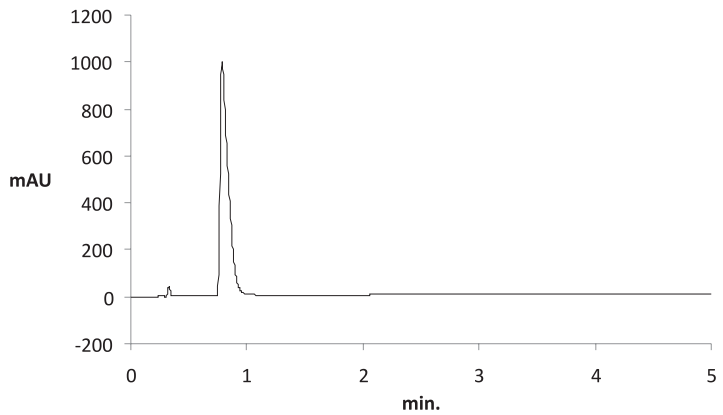


Figure 2. The HPLC chromatogram of irradiated CTD ($t_R$ = 0.787 min) at doses of 25 kGy (recommended for sterility)

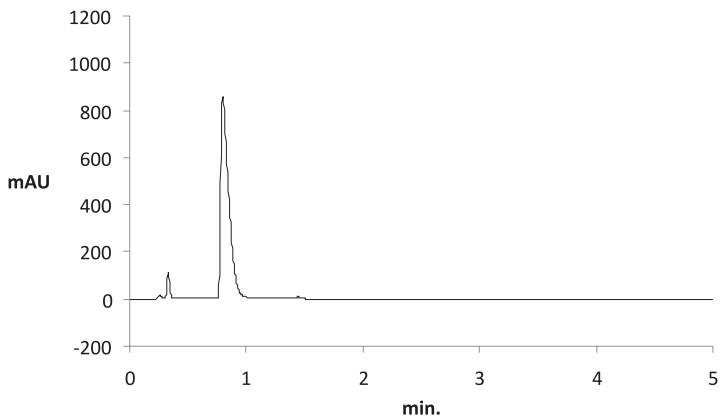


Figure 3. The HPLC chromatogram of irradiated CTD ($t_R$ = 0.787 min) at doses of 400 kGy

Table 1. Intra-day, inter-day precision (n = 6) and recovery studies (n = 6).

| Spiked concentration (mg/L) | Measured concentration ± S.D. (mg/L) | RSD (%) |
|---|---|---|
| Intra-day precision | | |
| 508.8 | 508.72 ± 0.02 | 0.0329 |
| 424.0 | 424.10 ± 0.06 | 0.0685 |
| 381.6 | 380.85 ± 0.04 | 0.0755 |
| Inter-day precision | | |
| 08.80 | 508.85 ± 0.07 | 0.0467 |
| 424.0 | 424.92 ± 0.08 | 0.0858 |
| 381.6 | 382.15 ± 0.06 | 0.1062 |
| Recovery studies | | |
| Spiked concentration (mg/L) | Measured concentration ± S.D. (mg/L) | Recovery (%) |
| 508.8 (~ 120%) | 509.11 ± 0.05 | 100.24 |
| 424.0 (~ 100%) | 423.95 ± 0.03 | 99.62 |
| 381.6 (~ 80%) | 381.59 ± 0.02 | 99.99 |

Table 2. Results of quantitative analysis of CTD before and after irradiation.

| Dose [kGy] | Content [%] |
|---|---|
| 0 | 100 |
| 25 | 97 |
| 50 | 94 |
| 100 | 93 |
| 200 | 91 |
| 400 | 80 |

matograms of CPS developed over a period of 0 to 5 min the following compounds were eluted: CTD with a retention time of 0.787 min and degradation product with retention time of 0.33 min. Peaks were symmetrical, clearly separated from each other (Figs. 2 and 3). The calibration plots were linear in the following concentration range 42.4–508.8 mg/L (n = 11, r = 0.9999). The calibration curve was described by the equation y = *a*x; y = (0.404 ± 0.002) x. The *b* value, calculated from equation y = *a*x + *b*, was not significant. Statistical analysis using Mandel's fitting test confirmed linearity of the calibration curves. The intra-day and inter-day precision values of measured concentration of CTD, as calculated from linearity plots are given in Table 1. The RSD values were from 0.0329 to 0.1062% demonstrating that the method was precise. The LOD and LOQ were 2.5 and 7.5 mg/L, respectively.

In contrast to the considerable instability of CTD under the influence of acid-basic hydrolysis (2), increased temperature and relative air humidity (3), after exposure to a radiation dose of 25 kGy CTD only 3% from initial concentration of CTD degraded (Table 2). A comparison of HPLC study results for non-irradiated and irradiated CTD samples demonstrated that the concentration of CTD changed after irradiation in higher doses (Table 2).

The effectiveness of radiation sterilization and possible changes in microbiological activity were also evaluated. The results of microbiological studies proved that irradiation at 25 kGy ensured the sterility of CTD samples. All tested species of reference strains showed sensitivity to CTD, both when non-sterilized and following different irradiation doses (Table 3). By comparing the activity of non-irradiated and irradiated CTD samples it was found that no significant differences between MIC values for the reference Gram-positive strains (*Listeria monocytogenes*, *Clostridium butyricum* and *Clostridium pasteurianum*) and Gram-negative *Klebsiella pneumonia* (Table 3) occur. On the other hand, CTD is active against *Listeria monocytogenes* and *Klebsiella pneumonia* only at high concentrations (Table 3). In the case of the reference strains (*Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*), the bactericidal properties of irradiated CTD samples were substantially reduced (Table 3). For *Staphylococcus aureus* even no inhibition of growth was observed. Among all the analyzed

Table 3. MIC values (ěg/mL) of irradiated CTD samples.

| Reference strain | CTD 0 kGy | CTD 25 kGy | CTD 400 kGy |
|---|---|---|---|
| Gram-negative bacteria | | | |
| *Salmonella enteritidis* ATCC 13076 | 0.24 | 32 | 32 |
| *Salmonella typhimurium* ATCC 14028 | 0.24 | 32 | 32 |
| *Escherichia coli* ATCC 25922 | 1 | 8 | 32 |
| *Klebsiella pneumoniae* ATCC 31488 | 32 | 32 | > 256 |
| *Pseudomonas aeruginosa* ATCC 27853 | 64 | 128 | 256 |
| *Proteus vulgaris* ATCC 33420 | 0.12 | 1 | 4 |
| Gram-positive bacteria | | | |
| *Clostridium butyricum* ATCC 860 | 4 | 4 | 16 |
| *Clostridium pasterianum* ATCC 6013 | 4 | 4 | 16 |
| *Staphylococcus aureus* ATCC 25923 | 8 | > 256 | > 256 |
| *Listeria monocytogenes* ATCC 7644 | 64 | 64 | 256 |

strains of the reference bacteria *Proteus vulgaris* showed the highest sensitivity to CTD, even in the variant, in which the irradiation dose applied on the investigated compound was 400 kGy. The difference connected with the sensitivity of Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive bacteria (*Clostridium* sp.) could have resulted from the differences connected with cell wall structure, particularly since the bactericidal activity of CTD results from the inhibition of bacterial cell wall synthesis. Moreover, as a result of the effect of radiation sterilization, the spatial conformation of the compound could have changed and this may affect its activity towards the tested bacterial species. Taking into account differences between the sensitivities of HPLC and microbiological methods, the evaluation of CTD susceptibility to degradation was further studied at higher doses of 50, 100, 200 and 400 kGy. The CTD degradation rate increased with a radiation dose. Based on the HPLC analysis, a decrease in the CTD concentration connected with increasing radiation and the appearance of peaks originating from radiolysis products were observed (Figs. 2 and 3).

## CONCLUSIONS

The isocratic HPLC method developed for the analysis of CTD in pharmaceutical preparation is selective, precise and accurate. The method is useful for routine analysis due to short run time and low amounts of used solvents (acetonitrile) in mobile phase. Microbiological methods, as they are more selective, should support HPLC methods for CTD quality control. The susceptibility to radiation of CTD limits the application of radiation sterilization as an alternative method to obtain sterile dosage forms of CTD. Changes in the concentration of CTD irradiated at 25 kGy cause a decrease in its microbiological activity against Gram-negative strains. Although that dose of radiation does not lead to any significant differences in the assay of CTD (3%), its bactericidal activity against *Salmonella enteritidis*, *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris* was decreased.

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