Photodegradation Kinetics of Sodium Ceftiofur in Aqueous Solution Determined by LC Method

M. J. e Souza[1,2], P. S. Souza Filho[3], A. I. H. Adams[4] and A. M. Bergold[1,*]

[1]*Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul - UFRGS. Av. Ipiranga, 2752, CEP 90610-000, Porto Alegre, RS, Brazil*

[2]*CTEFAR - Centro de Desenvolvimento de Testes e Ensaios Farmacêuticos - Universidade Federal de Santa Maria - UFSM, CEP 97105-900, Santa Maria, RS, Brazil*

[3]*Programa de Pós-Graduação em Engenharia Agrícola, UFSM, CEP 97105900, Santa Maria, RS, Brazil*

[4]*Departamento de Farmácia Industrial, UFSM, CEP 97105-900, Santa Maria, RS Brazil*

Abstract: The stability of the broad-spectrum antibiotic ceftiofur was studied in order to investigate the kinetics of photodegradation of this drug, using a stability-indicating liquid chromatography (LC) method. The degradation was carried out in aqueous solutions, prepared from reference substance and two pharmaceutical products (Excenel® and Topcef®) containing sodium ceftiofur powder for injection, in quartz cells under UVC (254 nm) and UVA light (352 nm). The kinetics parameters of reaction order and the rate constants of the degradation were determined for both products. The degradation process of sodium ceftiofur in solution can be described by first-order kinetics under the experimental conditions used in this study. The obtained results show that the LC method is satisfactory in the determination of the kinetics of degradation of sodium ceftiofur in the presence of its photolytic degradation products. The present study reveals the photolability of the drug. Thus, appropriate light protection is recommended during the storage and handling of products containing sodium ceftiofur.

Keywords: Column liquid chromatography, Ceftiofur stability, Kinetic of degradation, Sodium ceftiofur.

INTRODUCTION

Photochemical stability of pharmaceutical substances is a matter of great interest, both for analytical and for practical purposes. Drug light-induced degradation can result in a decreased efficacy and sometimes also involve significant adverse side effects after drug administration [1-3].

Sodium ceftiofur is a third generation broad-spectrum cephalosporin, formulated as an intramuscular injection which is used to treat respiratory diseases in swine, ruminants and horses. Ceftiofur is effective against a wide variety of Gram-positive and Gram-negative microorganisms [4].

Chemically ceftiofur (Fig. **1**) is the (6R-(6α, 7β (Z)))-7-(((2-furanylcarbonyl)-thio) methyl)-8-oxo-5-thia-1-azabicyclo (4.2.0) oct-2-ene-2-carboxylic acid [5]. This compound is very susceptible to acid, alkaline, and enzyme-catalyzed hydrolysis, producing a number of unstable degradation products. Hydrolysis of ceftiofur is complex; it is readily hydrolyzed to desfuroylceftiofur and is further converted into more complex products [6] such as the dimer of desfuroylceftiofur, corresponding thiolactone and various other forms. The influence of pH and temperature on kinetics of ceftiofur degradation in aqueous solutions have been early described [7].

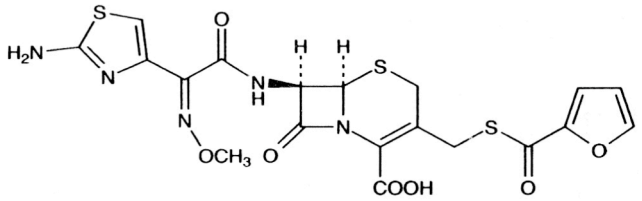

Fig. (1). Chemical structure of sodium ceftiofur.

Preliminary stability investigations realized by our research group revealed that ceftiofur sodium undergo degradation upon exposure to light and its photolability was established by forced degradation tests (stress testing).The International Conference on Harmonization (ICH) guideline presents the standard conditions for photostability studies and requires that stress testing must be carried out to elucidate the inherent stability characteristics of the active substance in a pharmaceutical preparation. Following the recommendations of this guide, the light testing should be an integral part of stress testing. Besides, a stability-indicating method is necessary to quantify the drug in the presence of its degradation products (DP), and the method should be capable to resolve and detect photolytic degrading products which can appear during the study [8].

*Address correspondence to this author at the Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul - UFRGS. Av. Ipiranga, 2752, CEP 90610-000, Porto Alegre, RS, Brazil; Tel: 0055 51 33085451; Fax: 0055 51 33085313;
E-mail: ana.bergold@ufrgs.br

1876-5181/10 2010 Bentham Open

Light-stability testing of pharmaceutical formulations should provide information related to the practical use of the product. Photostability testing according to the ICH guideline will give an indication as to whether photochemical degradation of the drug substance or drug product is likely to occur during the shelf-life. The results are used to make labeling decisions. Based on data related to sunlight conditions, it is also possible to make recommendations on how the product should be handled in use.

The present work was aimed to investigate the photostability of sodium ceftiofur in solution, under forced exposure to UVC and UVA radiations and determine its photodegradation kinetics, using a selective and reliable liquid chromatographic method (LC) previously developed and validated according to ICH guideline [9].

MATERIAL AND METHODS

Samples

The sodium ceftiofur reference substance (assigned purity 101.2%) was obtained from Orchid Chemicals& Pharmaceuticals Ltd. (Chennai, India) and used without purification. Pharmaceutical products Excenel® (Pharmacia & Upjohn Company, Kalamazoo – Michigan – U.S.A.) and Topcef® (Eurofarma Lab. Ltda – Brazil) containing sodium ceftiofur were obtained commercially and claimed to contain 1 g of sodium ceftiofur powder/flask.

REAGENTS AND SOLVENTS

All used chemicals were of pharmaceutical or special analytical grade. Acetonitrile was HPLC grade (Tedia®, USA). Analytical reagent grade di-sodium hydrogen phosphate dihydrate and orthophosphoric acid were purchased from Merck, Darmstadt, Germany. Water HPLC grade obtained from Milli-Q RO system, was used.

INSTRUMENTATION AND ANALYTICAL CONDITIONS

The developed method was performed with a LC system consisting of a Shimadzu® SCL-10AVP system equipped with a Shimadzu model LC-10 ADVP pump, and SPD 10AVP, variable-wavelength UV-VIS and SPD-M-10A VP DAD detector a SCL-10A system controller, and a SIL-10 A VP auto injector, (Shimadzu, Kyoto, Japan). The detector was set at 292 nm and peak areas were integrate automatically by computer using a Class VP® software program. The method was carried out on a pre-packed, Lichrospher® C_{18} (250 mm x 4,6 mm i.d. 5 µm particle size) column from Merck (Germany). The mobile phase was a mixture of 0.02M di-sodium hydrogen phosphate dihydrate buffer (pH 6.0), adjusted by addition of orthophosphoric acid 85% and acetonitrile (78:22, v/v). The injection volumes were 20 µL and eluted at a flow rate of 1.0 mL min^{-1}.

The sensitivity was 1.0 AUFS. The HPLC system was operated at room temperature (23 °C ± 1 °C). The mobile phase was filtered through a 0.45 µm membrane filter and degassed with a helium sparge for 15 min. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

PHOTODEGRADATION CONDITIONS

The photostability of the drug was studied with two light sources: UV fluorescent lamp model (*Light express LE,* 30W), emitting radiation at 254 nm (UVC light) and Black-light blue lamp (*Orion,* 30 W), emitting radiation at 352 nm, (UVA light), both fixed into a chamber, in a horizontal position. The internal surface of the chamber was coated with mirrors, in order to distribute the light uniformly. The effect of the light was studied exposing the aqueous sample solutions in 1 cm quartz cells, in a distance of 8 cm of the lamp each one. The temperature inside the chamber was monitored.

DEGRADATION STUDY

The kinetics of photodegradation of sodium ceftiofur was evaluated in water. Stock solutions (1mg mL^{-1}) were prepared from the reference substance, Excenel® and Topcef®. The stress degradation study was performed exposing the solutions contained in quartz cells in the chamber. Different time intervals were employed according the light source: 0, 30.0, 60.0, 90.0 and 120.0 min for UCV radiation and 0, 1.0, 3.0, 16.0 and 24.0 h for UVA radiation. In order to evaluate the contribution of thermally induced change to the total change, protected samples, wrapped with aluminum foil, were used as dark controls. Three samples were analyzed at each time interval; after exposition, they were diluted with the mobile phase to give final concentration of 60 µg mL^{-1} and assayed by HPLC. The stock standard solutions were prepared in water and diluted with mobile phase to the concentration of 60 µg mL^{-1}. All solutions were injected in triplicate.

KINETICS CALCULATIONS

The degradation rate kinetics of sodium ceftiofur was determined by plotting concentration of the remaining drug versus time (zero-order process), log of concentration of the drug versus time (first-order process), and reciprocal of concentration of the drug versus time (second-order process). The regression coefficients (*r*) were obtained, and the best observed fit indicates the reaction order. The kinetics parameters like apparent order degradation rate constant (*k*), and t_{90} (time where 90% of original concentration of the drug is left) were obtained.

The kinetics model can be represented as:

$$C = C_o - k.t$$

t 90% = 01 C_o / k (zero-order reaction)

$$ln\,C = ln\,C_o - k \cdot t \therefore C = C_o \cdot e^{-k \cdot t}$$

t 90% = (0.106) / k (first-order reaction)

$$\frac{1}{C} = \frac{1}{C_o} + k \cdot t \therefore C = \frac{C_o}{1 + C_o \cdot k \cdot t}$$

t 90% = 1/9 k C_o (second-order reaction)

Equations

Where C_o is the concentration of the reactants under consideration at zero time, C is the concentration after reaction time t and k is the reaction rate constant.

RESULTS

The LC method previously developed and validated for the quantitation of sodium ceftiofur in pharmaceutical dosage forms [9] was applied in this study, since it could effectively separate the drug from its degradation products (DP). The chromatographic parameters of resolution, theoretical plates, and asymmetry were evaluated for sodium ceftiofur and for the main observed DP. Through the obtained results, the LC method can be satisfactorily employed as a stability-indicating method.

The exposition of sodium ceftiofur solutions to UVC and UVA light sources resulted in the data showed at Tables **1** and **2**.

Typical chromatograms, presenting the observed changes during the degradation under UVC and UVA light sources in comparison to the initial sample are demonstrated in

Table 1. Results of the Residual Concentration of Ceftiofur Sodium SQR, Excenel® and Topcef® in Aqueous Solutions after Photodegradation (UVC), using the LC Method

Sample	Time (min)	Sample Concentration (μg mL^{-1})	Measured Concentration[a] (μg mL^{-1})	Purity[a] (%)	RSD
SQR	0	60	60.00	100.00	0.18
	30		28.13	46.88	0.33
	60		16.98	28.30	0.64
	90		6.98	11.64	0.45
	120		2.88	4.38	0.59
Excenel®	0	60	60.34	100.56	0.22
	30		30.99	51.37	0.49
	60		19.79	32.80	0.71
	90		8.67	14.38	0.26
	120		2.76	4.60	0.64
Topcef®	0	60	60.14	100.23	0.24
	30		29.21	48.58	0.47
	60		18.93	31.47	0.39
	90		6.52	10.85	0.64
	120		2.94	4.89	0.44

[a]Mean of three analysis.

Table 2. Results of the Residual Concentration of Cefiofur Sodium SQR, Excenel® and Topcef® in Aqueous Solutions after Photodegradation (UVA), using the LC Method

Sample	Time (h)	Sample concentration (μg mL^{-1})	Measured concentration[a] (μg mL^{-1})	Purity[a] (%)	RSD
SQR	0	60	60.00	100.00	0.18
	1		51.30	85.50	0.36
	3		47.52	79.20	0.42
	16		43.53	72.56	0.29
	24		26.89	44.83	0.53
Excenel®	0	60	60.34	100.56	0.22
	1		50.88	84.80	0.47
	3		47.13	78.55	0.28
	16		43.56	72.60	0.45
	24		27.44	45.74	0.31
Topcef®	0	60	60.14	100.23	0.24
	1		51.12	85.20	0.19
	3		48.57	80.95	0.28
	16		45.70	76.18	0.52
	24		29.70	42.84	0.46

[a]Mean of three analysis.

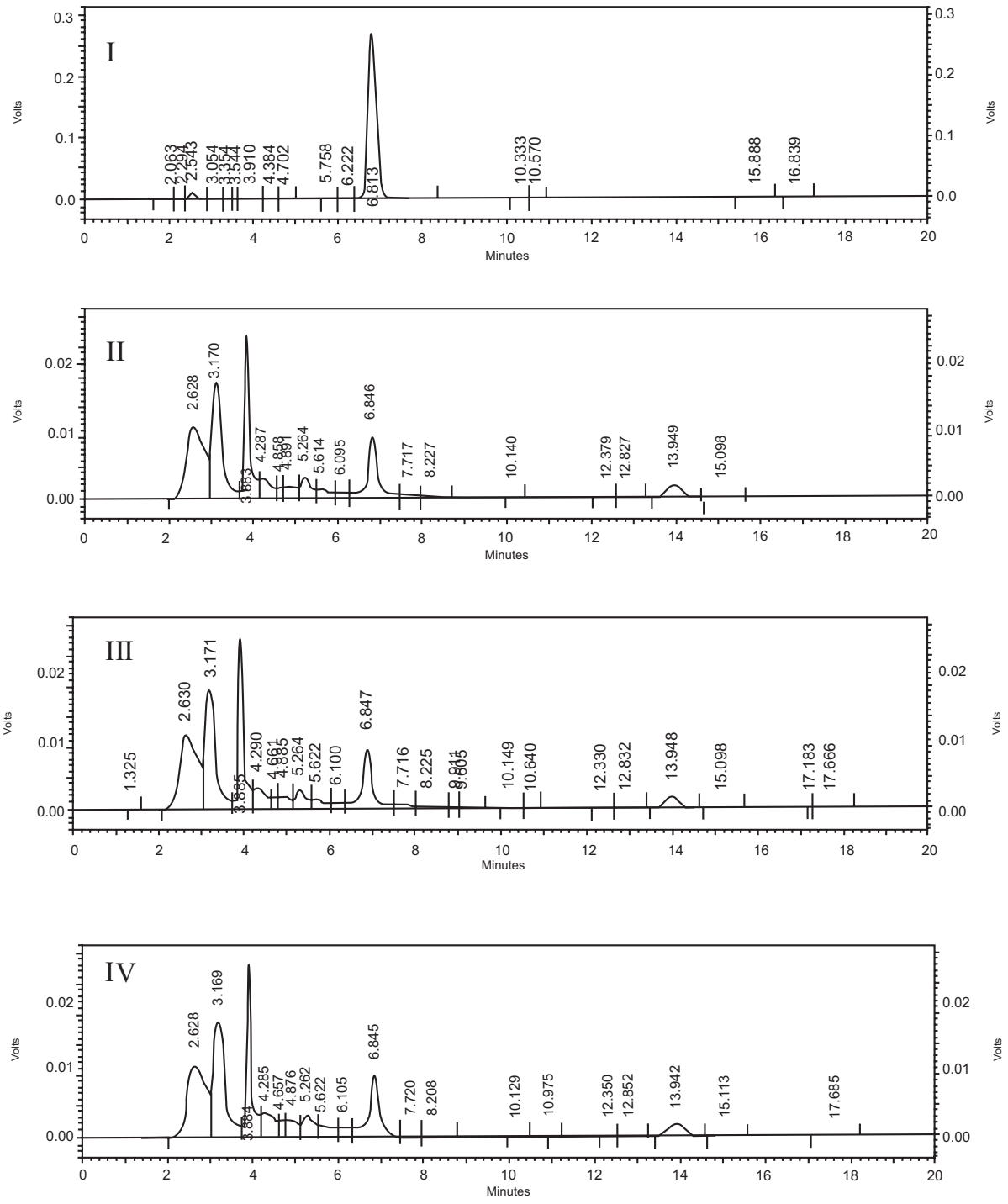

Fig. (2). Chromatograms of sodium ceftiofur RS not exposed in the time 0 (I), and exposed (II) to UVC radiation, Excenel® (III) and Topcef® (IV), exposed to UVC radiation, in aqueous solution after 120 min of irradiation. Chromatographic conditions as described in the text.

Figs. (**2**) and (**3**), respectively. It was observed that samples developed yellowish color after exposition to UVC light. Many degradation products are formed in this condition, being the peaks at around 2.6, 3.1, 3.8 and 13.9 min the major ones (Fig. **2**).

Under exposition to UVA light source, two main degradation product peaks (at 3.9 and 13.6 min) were detected, as is demonstrated on Fig. (**3**). It was observed that UVA light promoted a softer decrease on ceftiofur content.

The photodegradation kinetics was calculated for RS, Excenel® and Topcef®, through the fall in the drug content with the time. The concentration of remaining sodium ceftiofur was calculated at each time interval for the three replicates, in comparison with the mean concentration of the

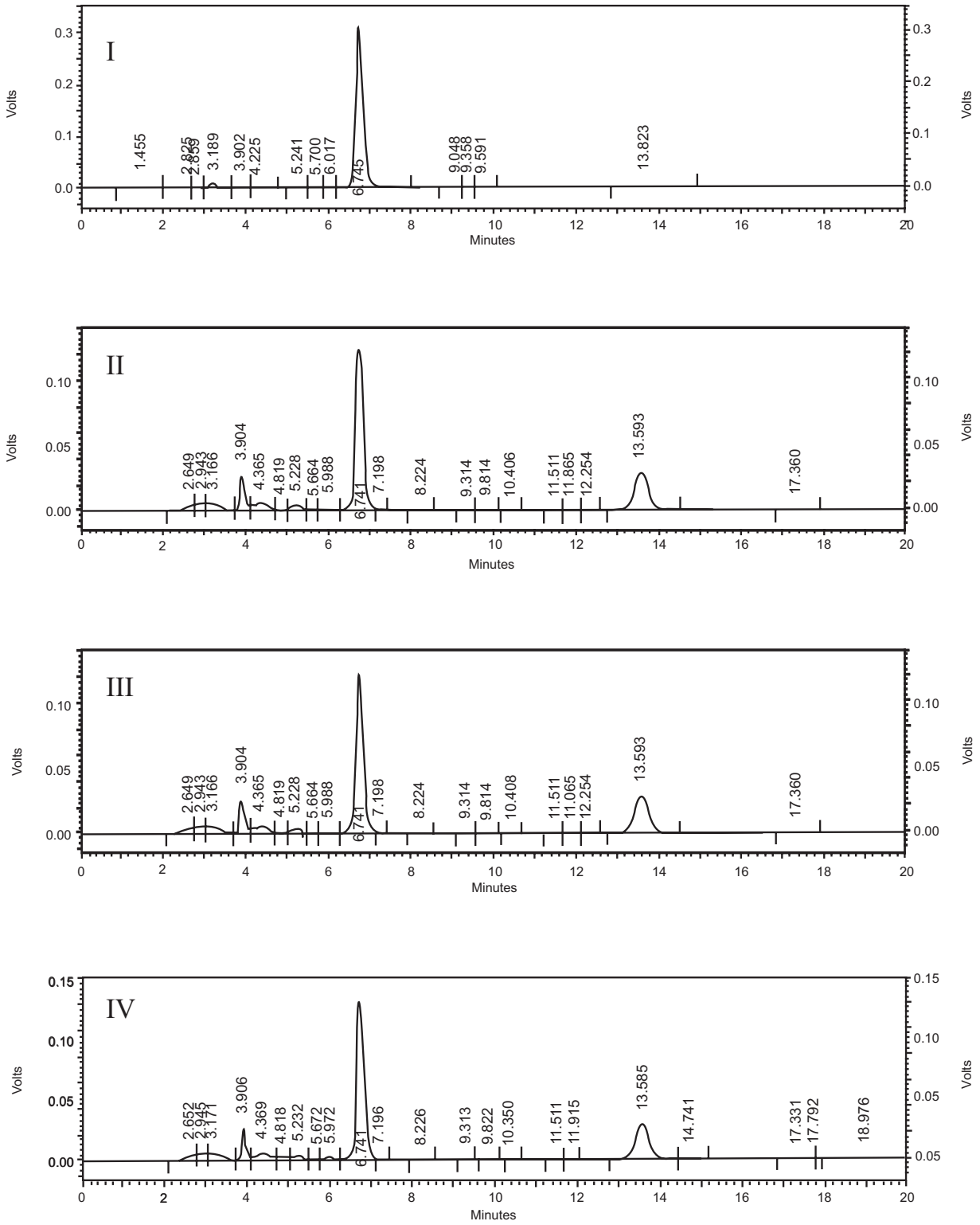

Fig. (3). Chromatograms of sodium ceftiofur RS not exposed in the time 0 (I), and exposed (II) to UVA radiation, Excenel® (III) and Topcef® (IV), exposed to UVA radiation, in aqueous solution after 24 h of irradiation. Chromatographic conditions as described in the text.

standard solution of the drug in each product. To determine the reaction order, first the data was plotted as established for each kinetic model. Then, the best coefficient of correlation indicated the order of the reaction. In this study, for both light sources, the degradation reaction can be described by first-order kinetic under the experimental conditions used. The plots of log of concentration of drug remaining versus time obtained in the course of the kinetics studies UVC and UVA are shown in Figs. (**4**) and (**5**), respectively.

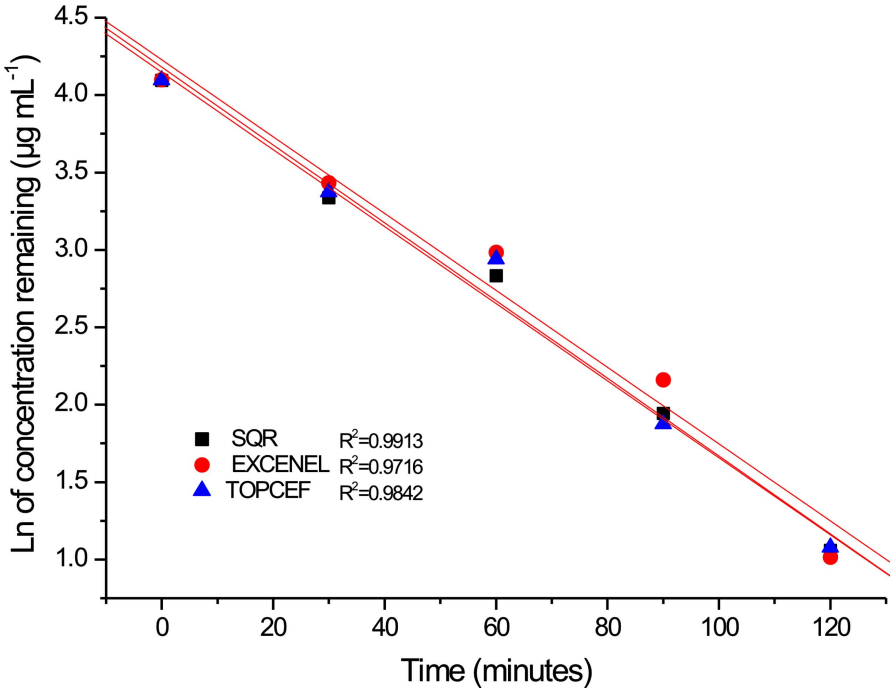

Fig. (4). First-order plots for the degradation of sodium ceftiofur RS, Excenel® and Topcef® irradiated with UVC (254 nm).

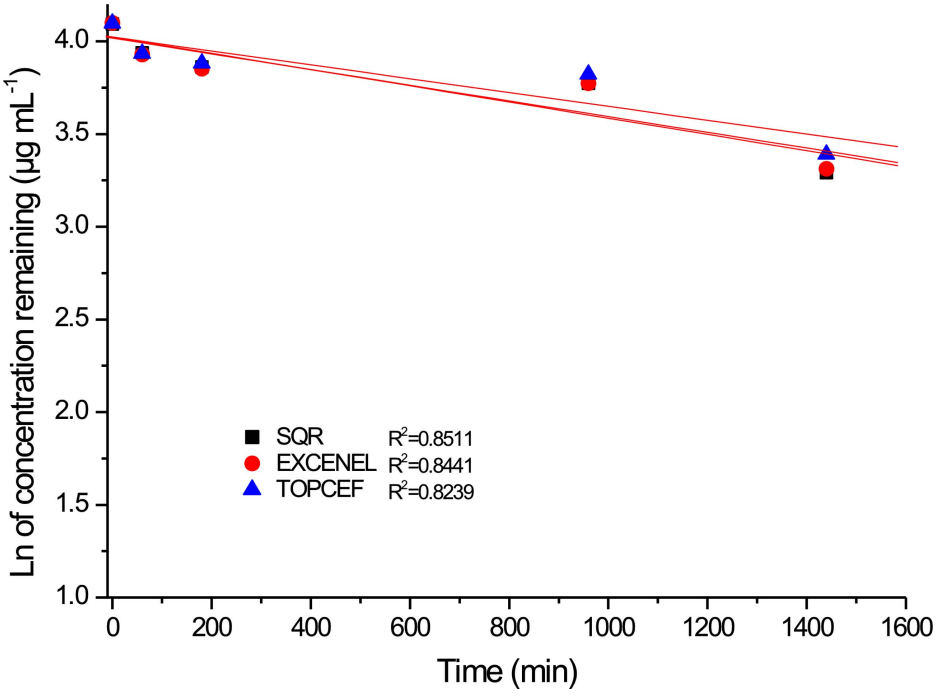

Fig. (5). First-order plots for the degradation of sodium ceftiofur RS, Excenel® and Topcef® irradiated with UVA.

From the slopes of the straight lines it was possible to calculate the first-order degradation rate constant*k*, and the $t_{90\%}$ for each sample tested (Table **3**).

DISCUSSION

Drug photostability constitutes an important current subject of investigation because the photodegradation process can result in a loss of the potency of the drug an also in adverse effects due to the formation of minor toxic degradation products [10]. Information about photostability of drugs can also help to determine the storage conditions of pharmaceutical products [3].

The results showed that UVC and UVA light promote a very fast decrease on sodium ceftiofur content, prepared as solution. It was observed that around 95% and 75% of the parent compound decomposed after a 2 h exposition to UVC

Table 3. Degradation Rate Constant *k* and $t_{90\%}$ for Ceftiofur SQR, Excenel® and Topcef® in Aqueous Solution after Photodegradation, Determined by LC Method

Sample	UVA		UVC	
	k (min^{-1})	$t_{90\%}$ (min)	k (min^{-1})	$t_{90\%}$ (min)
SQR	4.32	88.37	2.38×10^{-2}	4.39
Excenel®	4.60	83.11	2.20×10^{-2}	4.81
Topcef®	4.20	90.76	2.33×10^{-2}	4.55

and a 24 h exposure to UVA radiation, respectively. The stronger decrease of ceftiofur content under exposition to UVC light was expected, since it is more energetic. The controlled temperature into the chamber was always below 30 ºC, indicating that the DP observed were related just to light.

The degradation process of sodium ceftiofur in UVC and UVA light can be described by first-order kinetic under the experimental conditions used in this study. The $t_{90\%}$ for the samples tested indicate the strong susceptibility of sodium ceftiofur to light, even under the UVA wavelength.

The classic hydrolysis of the Δ3-cephem ring occurs in cephalosporins in aqueous solution, but it is obviously not sufficient to explain all these observations. On the other hand, our data corroborate the well-known yellowish coloration of cephalosporin during ageing as described in the literature. Lerner *et al*. [11] report competitive photolysis of cefotaxime consisting of at least two processes (one on the Δ3-cephem ring and the second on the methoxyimino group) which led to an intense yellowing of the solution corresponding to the destruction of Δ3-cephem ring and consequently loss of antimicrobial activity, reported by Rabouan - Guyon *et al*. [12].

CONCLUSION

The photodegradation kinetics of the antibiotic sodium ceftiofur RS, Excenel® and Topcef® by UVC and UVA light was determined. The photodegradation of sodium ceftiofur follow first-order reaction kinetics, in both light sources used. Ceftiofur was found to be photoreactive when exposed to UV radiation, consequently adequate light protection should be adopted for its storage and handling.

The kinetics parameters of degradation rate constant, and $t_{90\%}$ were calculated. The results obtained in this study indicate the photosensitivity of sodium ceftiofur RS and pharmaceuticals products (Excenel® and Topcef®) in solution exposed to UV light; extensive decomposition was observed. Consequently an appropriate protection is recommended during the storage and handling of this antibiotic after reconstitution.

ACKNOWLEDGEMENTS

The authors wish to thank Eurofarma Laboratories Ltda, São Paulo, Brazil for providing the reference substance. This study was performed with the collaboration of Universidade Federal do Rio Grande do Sul - UFRGS and Universidade Federal de Santa Maria - UFSM.

REFERENCES

[1] Andrisano V, Gotti R, Leoni A, Cavrini V. Photodegradation studies on Atenolol by liquid chromatography. J Pharm Biomed Anal 1999; 21: 851-7.

[2] Albini A, Fasani E. In: Albini A , Fasani E, Eds. Drugs: photochemistry and photostability. Cambrigde: Royal Society of Chemistry 1998; pp. 1-73.

[3] Moore DE. In: Tonnesen HH, Ed. Photostability of Drugs and Drugs Formulations. London: Taylor &Francis 1996; pp. 9-38.

[4] Hornish RE, Kotarski SF. Cephalosporins in veterinary medicine – ceftiofur use in food animals. Curr Top Med Chem 2002; 2: 717-31.

[5] The Merck Index. New Jersey: Merck & CO. INC. 2001.

[6] Koshy KT, Cazers AR. Controlled hydrolysis of sodium ceftiofur, a broad-spectrum cephalosporin; isolation and identification of hydrolysis products. J Pharm Sci 1997; 86: 389-95.

[7] Sunkara G, Navarre CB, Kompella UB. Influence of pH and temperature on kinetics of ceftiofur degradation in aqueous solutions. J Pharm Pharmacol 1999; 51: 249-55.

[8] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals (ICH). Validation of Analytical Procedures: Methodology. Geneva, Switzerland 1996.

[9] Souza MJ, Nogueira DR, Silva LM, Arend MZ, Souza FPS, Bergold AM. Development and validation of an HPLC method for determination of sodium ceftiofur. Chromatographia 2007; 65: 401-6.

[10] Kovariková P, Mokry M, Klimes J. Photochemical stability of nimesulide. J Pharm Biomed Anal 2003; 31: 827-32.

[11] Lerner DA, Bonnefond G, Fabre H, Mandrou B, Simeon D, Bouchberg M. Photodegradation paths of cefotaxime. J Pharm Sci 1988; 77: 699-703.

[12] Rabouan-Guyon SM, Guet AF, Courtois PY, Barthes DMC. Stability study of cefepime in different infusion solutions.Intern J Pharm 1997; 154:185-90.

Received: September 09, 2009 Revised: November 03, 2009 Accepted: November 08, 2009