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A stability study of tetracycline and tetracycline cyclodextrins in tablets using a new HPLC method

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Abstract

A sensitive, rapid, reproducible, easy and precise reverse-phase high-performance liquid chromatographic assay for stability studies of tetracycline hydrochloride (TC·HCl) formulated with different excipients and techniques without using gradient elution, extraction methods, and at ambient temperature has been developed and validated. The method was especially developed for the analysis of TC·HCl and its main degradation product, 4-epi-anhydrotetracycline, due to its toxicity, in samples obtained from stability studies of solid dosage forms (tablets). The influence of the excipients used for the pharmaceutical design of the different tablet formulations and the use of hydroxypropyl-β-cyclodextrin on the stability were evaluated. A significant improvement of the stability of TC·HCl was found in some tablet formulations. The precision and accuracy of the method was also studied for the encapsulated TC·HCl, and no significant interferences were found. The results obtained suggested that the developed HPLC method is selective and specific for the analysis of TC·HCl samples, and that it can be applied for long-term and accelerated protocols for stability studies.

Keywords: Tetracycline HCl; 4-Epi-anhydrotetracycline; Cyclodextrins; Performulation; Stability

1. Introduction

The group of tetracyclines provides a wide range of antimicrobial activity that overlaps that of many other antimicrobial drugs. They have been used extensively both for the treatment of infectious diseases and as an additive to animal feeds to facilitate growth. Both uses have resulted in increasing bacterial resistance to these drugs. Although the development of new antimicrobial agents that are more effective for specific infections and less toxic have declined the indications for its use, tetracyclines are still widely used in both human and veterinary pharmaceutical formulations.

Some degradation products of tetracyclines can appear when tetracycline hydrochloride (TC·HCl)

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is stored under adverse conditions, especially light, high temperatures and humidity. It is known that some degradation reactions can also take place in the stomach, with a reversible formation of epimers at position C_4 like 4-epitetracycline in weak acid conditions and anhydrotetracycline in strong acidic conditions, due to conformational changes in the ring system, epimerization at carbon-4 or dehydration and aromatization of the C ring, respectively [1] (Fig. 1). Besides, the presence of a hydroxyl group at position 6 increases the dehydration conformational change [2]. In a final step, both reactions can lead to the formation of 4-epi-anhydrotetracycline (4-EATC). The aforementioned structural changes also provoke a modification of the biological activity. In this way, the toxic effects of 4-EATC have been attributed to the relative position of the dimethylamino group at position 4 with respect to the rest of the molecule [3]. Due to its toxicity, the limit for 4-EATC in those pharmaceutical dosage forms containing tetracyclines is described in the Pharmacopeiae: no more than 3.0% of the dosage in solid form (tablets) is the limit established by the United States Pharmacopeia [4].

In the present paper, a preformulation study leading to the development of new TC·HCl tablet formulations, where its stability was increased by using technological strategies like complexation with hydroxypropyl-β-cyclodextrin (HP-βCD), has been carried out. Some accelerated stability studies were conducted under different stress conditions (humidity–temperature and radiations) in order to elucidate the effect of formulation on the stability of TC·HCl. A reverse-phase high-performance liquid chromatographic (HPLC) method

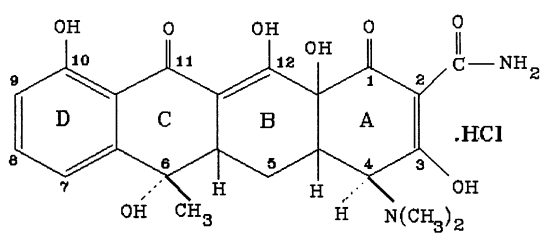

Fig. 1. Chemical structure of tetracycline HCl.

for the analysis of the TC·HCl and 4-EATC have been developed and validated. Its suitability for such analysis in the frame of the aforementioned stability studies was also proved.

The method described in this article is simple and provided good selectivity and resolution between the mentioned substances without increasing the temperature or using a gradient elution and avoiding the use of extraction methods in contrast with other analytical methods described, not only by different authors [5–9], but also by some Pharmacopeiae [4,10]. Spectrophotometric detection in HPLC is generally regarded as being more usefully and less technically demanding than other techniques. Nevertheless, some of the reported methods use HPLC with different detection techniques like amperometry [11], electrochemistry [12] or spectrofluorimetry [2,13,14].

2. Materials and methods

2.1. Materials

TC·HCl (USP 23 grade) was supplied by COPANOR S.A. (Spain). 4-EATC reference standard and HP-βCD ENCAPSIN HPB® were purchased from Janssen Biotech. N.V. (Olen, Belgium). The selected direct compression diluent was CELLACTOSE®, a commercially available cellulose–lactose complex (CLC) 25/75%, which was kindly supplied by Meggle (Forum Chemicals, Redhill, UK). Sodium Starch glycolate (SSG) EXPLOTAB® (Edward Mendell Co. Inc., Julia Parrera S.A., Barcelona, Spain) was used as a disintegrant. Magnesium stearate (Panreac-Montplet, Barcelona, Spain) was chosen as lubricant. All HPLC solvents and reagents were of analytical grade. Milli-Q water was used throughout all the experiments. The internal standard *p*-hydroxybenzoic acid was obtained from Sigma Chemical Co. (St Louis, MO, USA).

2.2. Preparation of physical mixtures and tablets

A series of physical mixtures were prepared with accurately weighed amounts of TC·HCl (2.6 g) and HP-βCD (1.0 g) in a molar ratio (2:1) by

Table 1
Composition of the different tetracycline hydrochloride tablet formulations (%)

Compound	Formulation			
	I	II	III	IV
TC·HCl	–	–	27.77	27.77
TC·HCl/HP-β CD	27.77/36.11	27.77/36.11	–	–
CLC	32.11	32.11	68.23	68.23
SSG	2	–	2	–
Mg stearate	2	2	2	2

simple pulverization and mixing of both solids at room temperature. Preliminary studies showed that TC·HCl was strongly coloured with a liophyllization process, which indicates an evident degradation; because of this fact, our mixtures were then frozen during 48 h at −20°C.

Both physical mixtures of TC·HCl/HP-βCD and TC·HCl alone were appropriately mixed in a V-mill (type MV-6; Turu S.A., Tarrasa, Spain) with the excipients of each formulation. The tablets were prepared by direct compression having a theoretical weight of 450 mg, containing 125 mg TC·HCl or 287.5 mg TC·HCl/HP-βCD physical mixture (equivalent to 125 mg TC·HCl), using an automatic eccentric tableting machine (Bonnals B-40, Barcelona, Spain), equipped with 12 mm diameter plain punches. All tablet formulations designed are fully described in Table 1.

2.3. Solubility measurement

A constant but excess amount of TC·HCl (0.05 M) was added to several HP-βCD aqueous solutions having increasing concentrations (2.5×10^{-3}, 5×10^{-3}, 7.5×10^{-3}, 1.0×10^{-2} and 2.5×10^{-2} M). These solutions were mixed by magnetic stirring at 500 r.p.m. at room temperature during 48 h. The mixture was them filtered through a 0.45 μm pore size filter and the drug concentration was determined by UV spectrophotometry at 356 nm using a Beckman DU-6 spectrophotometer (Beckman Instruments Inc., Fullerton, USA) with a previously validated method (unpublished data). It was observed that the presence of HP-βCD did not interfere in the spectrophotometric assay of TC·HCl. All the experiments were performed in triplicate.

2.4. Determination of the stability constant

The phase solubility diagram was prepared following the method described by Higuchi and Connors [15]. An apparent 1:1 stability constant, K (M^{-1}), was calculated from the slope (s) and intercept values (I) of the initial straight line portion of the solubility diagram, according to the following equation:

$$K = s/I(1 - s) \quad (1)$$

2.5. Thermal analysis

Differential scanning calorimetry (DSC) studies were carried out on a Perkin Elmer DSC AD-2Z/ Mettler DSC 20 differential scanning calorimeter. All measurements were performed at a heating rate of 10°C/min (over a temperature range of 50–250°C) under a nitrogen flow. Samples of about 15–20 mg were previously accurately weighed in hermetically sealed aluminium pans using a Metter M3 microbalance. The calorimeter was calibrated against pure indium and bismuth metal (melting points, 156.6 and 273.0°C, respectively).

2.6. HPLC analysis

TC·HCl and 4-EATC were assayed simultaneously using a reverse-phase HPLC method. The separation was performed on a 20 cm × 4.6 mm (10 μm I.D.) Lichrosorb RP-8 column (Hewlett Packard Co, Idaho, USA). The composition of the mobile phase was optimised using different proportions of 0.01 M oxalic acid (adjusted at pH 3 with ammonium hydroxide), methanol and acetonitrile to obtain a final proportion of 70:12.5:17.5. The solvents used were of HPLC grade (Lab-Scan; Analytical Sciences, Dublin, Ireland) and Milli-Q water was used thorough all the experiments. The mobile phase was always filtered through a 0.45 μm cellulose acetate filter under vacuum and degassed by ultrasonication (J.P. Selecta S.A., Barcelona, Spain).

A flow rate of 1.25 ml/min, in which resolution of peaks is enough, was selected. A HPLC system consists of a Gilson HPLC pump 305-306, and a Gilson UV spectrophotometric detector 116, fixed at $\lambda_{max} = 270$ nm was used. The samples were injected using a 231 XL Gilson auto-injector. Data acquisition was completed with a Spectra-Physics 4270 integrator, which calculated the sample concentrations of TC·HCl and 4-EATC using as a reference an internal standard (4-hydroxybenzoic acid) that was previously added to the samples.

All samples were prepared by placing a previously weighed tablet in a 10 ml tube with 10 ml Na_2-ethylenediamine tetraacetic acid aqueous solution 0.5% (w/v). After vigorous shaking, each sample was maintained in an ultrasonic bath for 10 min. Then, the sample was transferred to a 50 ml volumetric flask and diluted to volume with mobile phase. An aliquot of 6 ml of each solution was transferred to a new 50 ml volumetric flask and diluted with 10 ml internal standard solution, having a concentration of 50 μg/ml 4-hydroxybenzoic acid in mobile phase. All flask were filled with mobile phase to volume; 10 ml of each sample were centrifuged during 15 min at 1000 r.p.m., filtered through a 0.45 μm filter and transferred to a glass vial for the injection.

2.7. Relative humidity and temperature stability testing

TC·HCl stability studies were carried out under stress conditions using an accelerated testing that conformed to the requirements and specifications described in the ICH stability guidelines [16] and some Pharmacopeiae in the Mediterranean Climate Zone (zone II) [17,18].

The influence of humidity and temperature on the chemical stability of the TC·HCl in the tablets was studied under two storage conditions for the different formulations (I, II, III, and IV) designed (described in Table 1), $25 \pm 2°C/60 \pm 5\%$ relative humidity (RH) and $40 \pm 2°C/75 \pm 5\%$ RH, during 6 months.

The remaining amount of TC·HCl and the amount of 4-EATC formed were determined at 0, 0.5, 1, 2, 3, 4, 5 and 6 months using the already described HPLC method.

2.8. Photostability testing

The photodegradation of the TC·HCl tablets were studied using photon sources that simulated daylight or room light, thus reflecting manufacturing and dispensing process conditions. In our case, the main aim was to clarify the effect of the irradiated light on the photodegradation behaviour of TC·HCl tablets in the presence of HP-βCD. A fluorescent lamp (Svlvania F20 T 12/D, lifeline daylight, Germany) and a UV lamp (Svlvania 6TC 1.5W germicidal G15 T8, Germany) were selected in the present photodegradation study, following the ICH suggestions for photostability tests [16,19,20].

All tablets were appropriately placed in a suitable glass dish without cover and continuously exposed to light at a distance of 40 cm for 3 months. At each sampling time (7, 15, 30, 45, 60, 75 and 90 days), the surface of the tablets were examined looking for any change in their colour and in the homogeneity. The remaining amount of TC·HCl and the photodegradation products were determined simultaneously by the previously described HPLC method.

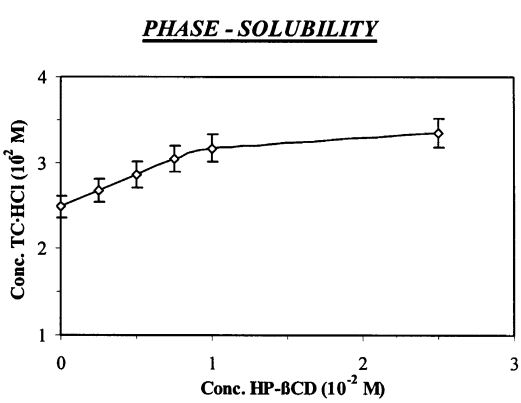

Fig. 2. Phase-solubility diagram (Higuchi–Connors).

3. Results and discussion

3.1. Phase solubility study and thermal analysis

Fig. 2 shows the phase solubility diagram of

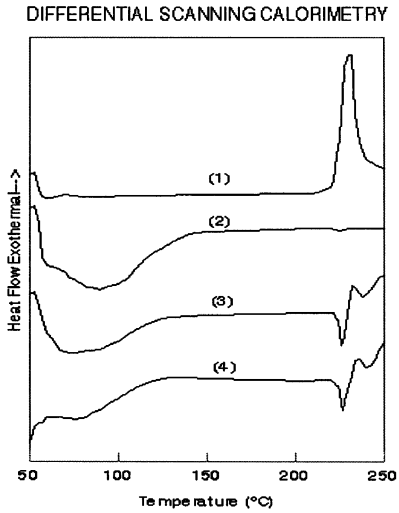

Fig. 3. Differential scanning calorimetry (DSC) thermograms corresponding to: (1) TC·HCl, (2) HP-βCD, (3) TC HCl/HP-βCD physical mixture, and (4) TC HCl/HP-βCD physical mixture + freezing at −20°C.

TC·HCl and HP-βCD in water. As can be seen, the solubility of TC·HCl was higher when the polymer concentration was increased, according to the A_N-type Higuchi–Connors phase solubility diagram [21]. The initial part of the diagram was employed to calculate the apparent stability constant, K, assuming a 1:1 molar complex. From the slope (0.748) and the intercept (0.02487) obtained from a linear regression analysis ($r^2 = 0.9998$), a value of $K = 119\ M^{-1}$ was obtained, which indicates a moderate affinity.

One possible explanation for the stability constant value of the [TC·HCl/HP-βCD] complex could be the high molecular weight of the drug (480.9 Da), which hindered the fitting of the molecule inside the HP-βCD cavity. According to Szetli [22,23], stable complexes can only be formed if the molecular weight of the compound is less than 400 Da.

DSC thermograms obtained under the same conditions for the intact TC·HCl, HP-βCD, the physical mixture and the physical mixture + freezing are shown in Fig. 3. It was observed that TC·HCl exhibited a characteristic exothermic peak at 235°C, without other peaks due to impurities. Reported melting ranges for TC·HCl in the literature are 230–235°C [4], or 214°C [24]. The melting point was also determined and an exact value of 235°C was found, which fits with the previously obtained thermogram.

Samples of HP-βCD exhibited a broad endothermic effect with onset at 85–90°C, which was attributed to dehydration process (2) [25]. In contrast, the interaction of TC·HCl with HP-βCD is accompanied by a disappearance of part of the exothermic peak and a new endothermic peak appeared. The variation of enthalpy of fusion (ΔH) of the physical mixture showed a tendency to decrease in comparison with the single TC·HCl sample containing the same amount of drug. This can be due to a partial inclusion of the molecule of TC·HCl in the solid state.

In general lines, first-order transitions like crystallisation, dehydration and decomposition provide exothermic plots, which means that a certain energy is supplied; the integration of the curve gives the energy involved in process (1). It is known that solid–solid phase transition may origin endothermic or exothermic plots but that it shows a characteristic inflexion point. In our case, plots (3) and (4) make up an example of this, showing an usual behaviour that takes place when the transition or decomposition did not occur at all, which could be due to a partial inclusion of the molecule, and the endothermic melting peak of the [TC·HCl/HP-βCD] complex is followed by an exothermic peak due to crystallisation or a decomposition process [26].

Second-order transitions can occur, for instance, due to the transformation of a certain polymorph into another, giving as a consequence a change in the specific heat. A glass transition might take place during galenic processes (tableting, milling) or along the storage time.

3.2. Chromatographic method

A chromatogram resulting from the injection of a standard preparation containing known concentrations near those obtained in the assay preparations of internal standard (1), TC·HCl (2) and

4-EATC (3), is shown in Fig. 4. As can be seen, separation between the different peaks was good enough for the analysis of samples in a relatively short period of time. Modification of different conditions like flow rate or changes of the mobile phase originated a significant increase of the time necessary to perform each analysis. The resolution between the TC·HCl and 4-EATC, calculated as described in United States Pharmacopeia [4], was 11.048.

A prospective validation protocol described by different authors [27–31] was applied for our HPLC method. Accuracy and precision were studied through the comparison of a series of replicates containing different known concentrations of TC·HCl and 4-EATC standard solutions, using as internal standard 4-hydroxy-benzoic acid. Cochran's G-test and Student's t-test ($P = 0.05$) revealed good accuracy and precision ($G = 0.555$, $t = 0.563$). An average recovery of $100.26 \pm 1.61\%$ was achieved for TC·HCl, with simulated samples having similar concentrations to those obtained from stability studies. The method showed good linearity for TC·HCl/internal standard in the range of concentrations of 200–350 μg/ml ($r^2 = 0.99412$, relative standard deviation (R.S.D.) for response factors = 1.64%) for TC·HCl and a fixed concentration of internal standard of 50 μg/ml. 4-EATC was also assayed through a comparison with the same internal standard, using in all the standard solutions the maximum concentration of the degradation product that could be found in our tablets following the specifications described by the United States Pharmacopeia [4].

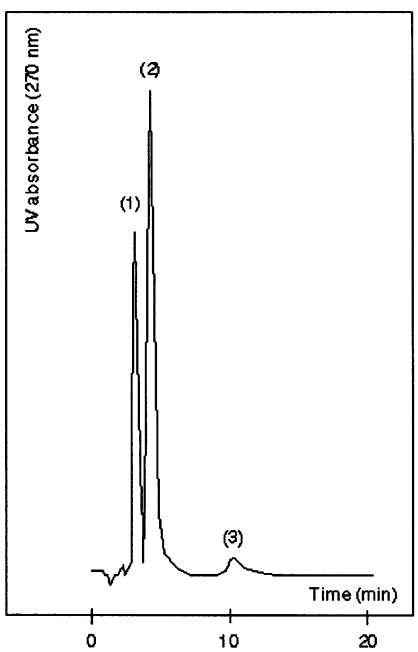

Fig. 4. Chromatogram showing the resulting peaks for: (1) internal standard (4-hydroxy-benzoic acid), (2) TC·HCl, and (3) degradation product (4EATC). Chromatographic conditions: 20 cm × 4.6 mm (10 μm I.D.) C-8 column; mobile phase, 0.01 M oxalic acid/methanol/acetonitrile (70:12.5:17.5); flow rate, 1.25 ml/min; wavelength, 270 nm.

Capacity ($K = 1.8181$), tailing factors ($T = 1.150$), quantification limit (Q.L. = 19.311 μ/ml), and detection limit (D.L. = 5.796 μg/ml), were calculated for TC·HCl as described in the European Pharmacopeia [10]. The good repeatability using four replicates (R.SD. = 0.96%) in the calibration is an indication of the good stability of these compounds during the chromatographic analysis.

The short time required for each analysis (< 15 min), the precision, and the high degree of sensitivity for the detection and quantification of low levels of degradation compound 4-EATC support the statement that the developed and validated HPLC method is appropriate for the analysis of TC·HCl and its degradate, 4-EATC, in solid dosage forms in the frame of stability studies.

3.3. Stability studies

The evolution of the degradation process of TC·HCl in the different tablet formulations under different conditions is graphically reported in Figs. 5–8 in terms of remaining drug in percentage versus time. Statistical analysis of such data was carried out by non-linear regression with weighted least-squares using the SIMFIT statistical package (W.G. Bardsley, 1998). Due to the lack of homogeneity in terms of goodness of fit using different kinetic models (such as zero order or first order) under each condition, a polynomial fitting was chosen for the whole comparison of all

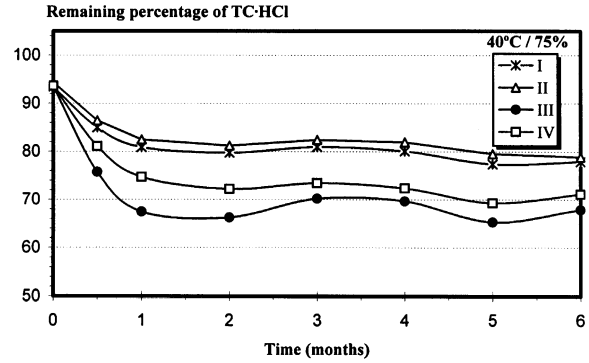

Fig. 5. Relative moisture–temperature stability diagram, 40°C–75% RH exposed conditions. *, Formulation **I**; △, formulation **II**; ●, formulation **III**; □, formulation **IV**.

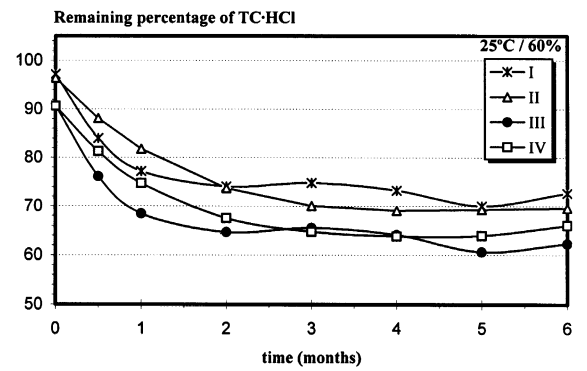

Fig. 6. Relative moisture–temperature stability diagram, 25°C–60% RH exposed conditions. *, Formulation **I**; △, formulation **II**; ●, formulation **III**; □, formulation **IV**.

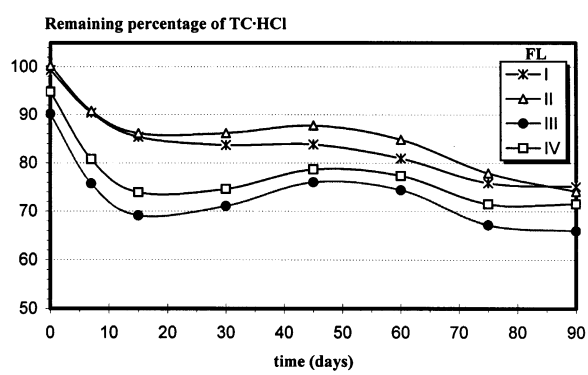

Fig. 7. Photostability diagram, fluorescent source exposed conditions. *, Formulation **I**; △, formulation **II**; ●, formulation **III**; □, formulation **IV**.

data. In this way, all plots were fitted using a fourth-order polynomial like $y = p_{(1)} + p_{(2)}t + p_{(3)}t^2 + p_{(4)}t^3 + p_{(5)}t^4$. Data resulting from each fitting are reported in Table 2. The shelf life or $t_{90\%}$, i.e. the time in which 10% was degraded and 90% was the remaining percentage of TC·HCl, was calculated in each case by mathematical extrapolation to the polynomial. Those values are also presented in Table 2.

The study of the plots obtained and the mathematical models suggested that the degree of degradation of TC·HCl was significantly lower in the presence of HP-βCD, and that the stability of TC·HCl was different after the exposition under high humidity and temperature or after the influence of a radiation source. It was observed that the effect of the radiation sources (UV, fluorescence) on the instability of the TC·HCl were greater than the degradation caused by humidity and temperature. Besides, the TC·HCl was relatively more sensitive to the UV source. It was also found that the change of colour from yellow to brown in the surface of the tablets was bigger after exposition to UV or fluorescence sources.

Regarding the influence of the other excipients, it was detected that the inclusion of sodium starch glycolate in the tablets had a negative influence on the stability of TC·HCl. In all cases, the presence of sodium starch glycolate (formulations I and III) provoked a significant decrease in the $t_{90\%}$ values, which is associated with a decrease in the stability of TC·HCl in the tablets. This can be due

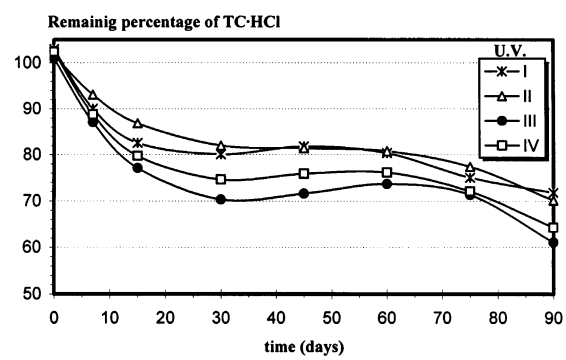

Fig. 8. Photostability diagram, UV source exposed conditions. *, Formulation **I**; △, formulation **II**; ●, formulation **III**; □, formulation **IV**.

Table 2
Shelf life ($t_{90\%}$) and polynomial parameters obtained from a non-linear regression analysis of stability data (remaining amount of TC·HCl detected versus time)

Storage conditions	Formulation	$p_{(1)}$	$p_{(2)}$	$p_{(3)}$	$p_{(4)}$	$p_{(5)}$	r^2	$t_{90\%}$ (days)
60% RH, 25°C	**I**	97.18	−34.47	18.08	−3.88	0.2851	0.754	7.080 ± 2.970
	II	96.41	−18.68	4.46	−0.426	0.0128	0.798	11.277 ± 2.090
	III	90.79	−38.04	19.48	−4.084	0.2937	0.626	0.630 ± 0.138
	IV	90.66	−22.12	7.27	−1.141	0.0715	0.652	0.900 ± 0.112
75% RH, 40°C	**I**	93.41	−22.42	12.38	−2.681	0.1948	0.512	5.013 ± 3.328
	II	94.39	−20.97	11.22	−2.347	0.1646	0.567	7.140 ± 3.210
	III	93.55	−47.70	27.02	−5.846	0.4248	0.655	2.310 ± 1.030
	IV	93.64	−32.97	17.51	−3.734	0.2712	0.738	3.510 ± 1.367
Fluorescent source	**I**	99.28	−1.662	0.0619	-9.34×10^{-4}	4.64×10^{-6}	0.883	7.408 ± 2.959
	II	100.20	−1.799	0.0727	-1.11×10^{-3}	5.40×10^{-6}	0.783	7.958 ± 2.681
	III	90.16	−2.793	0.1173	-1.77×10^{-3}	8.68×10^{-6}	0.511	0.056 ± 0.018
	IV	94.78	−2.677	0.1081	-1.61×10^{-3}	7.87×10^{-6}	0.746	1.933 ± 0.260
UV source	**I**	103.00	−2.384	0.0844	-1.18×10^{-3}	5.43×10^{-6}	0.865	7.047 ± 2.096
	II	101.70	−1.477	0.0381	-3.96×10^{-4}	1.25×10^{-6}	0.925	10.430 ± 2.333
	III	101.00	−2.398	0.0622	-5.90×10^{-4}	1.56×10^{-6}	0.877	5.293 ± 2.241
	IV	102.40	−2.413	0.0721	-8.49×10^{-4}	3.26×10^{-6}	0.844	6.199 ± 2.549

Table 3
Percentage of 4-EATC detected along the stability test under different temperature–relative humidity conditions[a]

Storage conditions	Formulation	Sampling time							
		0 months	0.5 months	1 month	2 months	3 months	4 months	5 months	6 months
75% RH, 40°C	**I**	N.D.	0.17	0.126	0.123	0.120	0.115	0.149	0.132
	II	N.D.	0.173	0.131	0.104	0.146	0.111	0.139	0.135
	III	N.D.	0.174	0.189	0.109	0.162	0.145	0.152	0.165
	IV	N.D.	0.191	0.180	0.146	0.138	0.150	0.155	0.169
60% RH, 25°C	**I**	N.D.	0.162	0.150	0.065	0.082	0.077	0.111	0.031
	II	N.D.	0.164	0.115	0.085	0.101	0.100	0.093	0.025
	III	N.D.	0.165	0.150	0.097	0.144	0.088	0.213	0.180
	IV	N.D.	0.183	0.174	0.109	0.167	0.188	0.201	0.120

[a] N.D., Not detected.

to the fact that the presence of such starch derivative as a disintegrant is normally associated with an increase in the hygroscopicity of the tablets, what could lead to an increase in the water content of the formulation promoting other degradation reactions.

The formation of the degradate 4-EATC along the stability studies under different conditions was also evidenced as exposed in Tables 3 and 4; in any case, the levels of 4-EATC obtained were not higher than the limit established by the Pharmacopeiae: no more than 3.0% of the declared amount of TC·HCl in the tablets [4].

4. Conclusion

With the described HPLC method, a good specificity and selectivity between TC·HCl and it's degradation product 4-EATC can be obtained.

Table 4
Percentage of 4-EATC detected along the stability test under different radiation conditions[a]

Storage conditions	Formulation	Sampling time							
		0 days	7 days	15 days	30 days	45 days	60 days	75 days	90 days
UV source	**I**	N.D.	N.D.	0.174	0.197	0.137	0.137	0.085	0.144
	II	N.D.	N.D.	0.117	0.117	0.094	0.121	0.093	0.124
	III	N.D.	N.D.	0.173	0.172	0.169	0.134	0.101	0.108
	IV	N.D.	N.D.	0.212	0.222	0.161	0.165	0.138	0.163
Fluorescent source	**I**	N.D.	N.D.	0.189	0.166	0.116	0.161	0.158	0.148
	II	N.D.	N.D.	0.140	0.129	0.158	0.166	0.159	0.131
	III	N.D.	N.D.	0.183	0.157	0.165	0.127	0.116	0.091
	IV	N.D.	N.D.	0.196	0.164	0.112	0.162	0.161	0.156

[a] N.D., Not detected.

Besides, the remaining amount of TC·HCl and the residue level of 4-EATC can be accurately determined under the aforementioned conditions. The reproducibility of the assay is also evident from the performed tests. Other advantages of the method (recovery and limits of detection and quantification) make it suitable for its application to stability studies in the described solid dosage forms.

The inclusion of HP-βCD has been found to be a good technological strategy to improve the stability of TC·HCl, even taking into account the large size of such a molecule. The results obtained in the present paper strongly suggest that the cyclodextrin could protect some specially labile radicals of the tetracycline molecule. Nevertheless, this statement should be prospectively studied with other techniques.

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