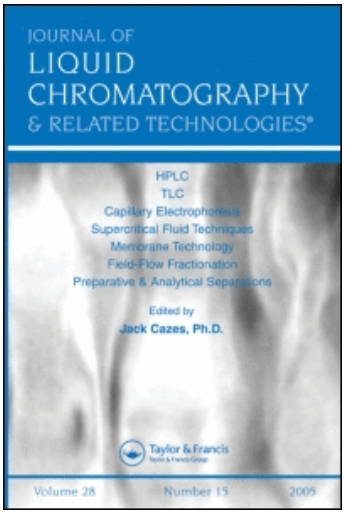

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SELECTIVE ASSAYS FOR QUANTITATION OF TENOXICAM IN PRESENCE OF ITS PHOTODEGRADATION PRODUCTS

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Online publication date: 10 January 2002

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JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
Vol. 25, No. 18, pp. 2821–2831, 2002

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ABSTRACT

Tenoxicam, a thienothiazine oxicam, is a potent anti-inflammatory and antirheumatic drug. A comprehensive study on the photostability of tenoxicam is presented, including a comparison of three different methods (HPTLC/densitometry, HPLC, CE) developed for the photostability testing of the title compound. The stability indicating capability of the respective assays is proven with sample solutions forcedly degraded by artificial irradiation from a xenon source. The chromatograms and the electropherogram of the resulting solution, show tenoxicam well resolved from the degradation products. The methods are applied for testing the photostability of solutions containing tenoxicam in various concentrations (2 mg mL^{-1}; 250 μg mL^{-1}; 40 μg mL^{-1}), and stored under different conditions. The stability of tenoxicam was found to be dependent on the nature of the light source, as well as on the concentration of the sample

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DOI: 10.1081/JLC-120014952
1082-6076 (Print); 1520-572X (Online)

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solution. The assays are validated and compared with respect to performance and precision.

Key Words: Tenoxicam; Antirheumatic drug; Photostability; HPTLC; HPLC; CE

INTRODUCTION

Tenoxicam [4-hydroxy-2-methyl-*N*-2-(pyridyl)-2H-thieno[2,3-e]1,2-thiazin-3-carboxamid-1,1-dioxide] is a potent anti-inflammatory and antirheumatic drug used in the treatment of chronic rheumatic disorders, such as rheumatoid arthritis, osteoarthrosis, and ankylosing spondylitis.[1] The stability of tenoxicam in aqueous media and in blood samples has been studied by HPLC showing a 50% loss of the substance after 3 h exposure to natural sunlight.[2] Other investigations show a 43% loss of tenoxicam in plasma after 3 h exposure to natural sunlight and a 100% loss after 6 h exposure.[3] Solutions stored in brown glass bottles have been proven to be stable for at least 24 h.[3] Investigations of the stability of the drug in aqueous media at 100°C at pH 1 and pH 10, showed tenoxicam to be more stable in basic than in acid solution; a quantitative HPTLC assay was used for the determination of tenoxicam.[4] Whereas, tenoxicam was found to be unstable when exposed to sunlight,[2,3] completely different data had been published concerning the photostability of the structurally related compound piroxicam. Piroxicam solutions (1% in aqueous media) exposed to sunlight were estimated to be stable for 72 h.[5]

These adverse results promoted interest to carry out a comprehensive study on the photostability of oxicams. Special emphasis should be laid on specifying the different factors influencing the extent and the rate of photodegradation of the compounds, since this has not been taken into account in the investigations published previously. We found the stability of piroxicam [4-hydroxy-2-methyl-*N*-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide], as well as of isoxicam [4-hydroxy-2-methyl-*N*-(5-methyl-3-isoxazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide], to be dependent of the nature of light; a marked concentration dependency was observed as well.[6,7] This concentration dependency of the photodegradation of the oxicams might help explain the inconsistent results published,[2,3,5] and points out the importance to consider and specify the various factors influencing the photostability of drugs. In this paper, a comparison of three different stability indicating methods (HPTLC/densitometry, HPLC, CE) developed for the photostability testing of tenoxicam, is presented. HPTLC/densitometry and CE are compared with HPLC, which currently still is the most frequently used method in drug analysis. The methods are validated and compared with respect to performance and precision. The stability of the drug

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solutions was investigated by quantitation of tenoxicam by the methods mentioned. The aim of the work was to study the extent of the influence of different factors (e.g., light source, concentration of sample solution) on the photodegradation rate of tenoxicam.

EXPERIMENTAL

Materials

The drug substance was obtained from Sigma (Vienna, Austria) and stored under light protection. Twenty-five percent NH_4OH solution analytical grade was obtained from Riedel-de Haen (Seelze, Germany). For HPTLC, dichloromethane, ethylacetate, and 96% acetic acid were of analytical grade and obtained from Merck (Darmstadt, Germany). Methanol HPLC reagent and water HPLC reagent were obtained from J. T. Baker (Deventer, Holland). Sodium acetate and conc. acetic acid for the preparation of acetate buffer pH 4.6, were of analytical grade. Tetrabutylammoniumhydrogensulfate (99%), for the aqueous component of the mobile phase, was obtained from Sigma-Aldrich (Steinheim, Germany). 20 mM sodium phosphate buffer solution pH 8.0 for HPCE was obtained from Fluka (Buchs, Switzerland).

Sample Preparation

Solutions containing tenoxicam at three different concentrations (2 mg mL^{-1}, 250 μg mL^{-1}, and 40 μg mL^{-1}) in 2.5% NH_4OH solution (pH ~11.8) were prepared. For each concentration, three sample solutions were prepared and each tested, in triplicate, for exposure to irradiation in the Suntest. For HPTLC and CE, the solutions were used as described; samples for HPLC were diluted with the eluent before injection (solutions of 2 mg mL^{-1} were diluted 1 : 50 v/v; of 250 μg mL^{-1} were diluted 1 : 10 v/v, and those of 40 μg mL^{-1} were diluted 1 : 2 v/v).

HPTLC/Densitometry

Equipment

Analyses were carried out using a Shimadzu CS-9301 PC Dual-Wavelength Flying Spot Scanner (P/N 206-80625). Separation was achieved on MERCK HPTLC plates 10 × 10 cm, silica gel 60 F_{254}, using a CAMAG horizontal developing chamber for 10 × 10 cm chromatographic plates. Plates are prewashed with methanol/dichloromethane 1 : 1 v/v before use. Bandwise sample application was performed with a CAMAG Linomat IV (Hamilton syringe 100 μL).

Analytical Conditions

The volume of the sample solution introduced to the HPTLC plate was chosen according to the concentration of each solution (1 μL for 2 mg mL^{-1}; 3 μL for 250 μg mL^{-1}; 10 μL for 40 μL mL^{-1}). HPTLC mobile phase was dichloromethane–ethylacetate–96% acetic acid (8/2/0.5 v/v/v).

The densitometric measurements were made at $\lambda = 280$ nm using zig zag scan with a swing width of 3 mm, a slit width of 0.4 mm, and a slit height of 0.4 mm. The comparison of the remission spectra of the tenoxicam peak of freshly prepared, as well as stressed solutions, proved that no degradation products overlap the peak of tenoxicam. For quantitation, external calibration was carried out. For each concentration range, five standard solutions were prepared. Linear calibration functions resulted for each concentration range of the samples [concentration 40 μg mL^{-1}: 44.0, 35.2, 26.4, 17.6, and 8.8 μg mL^{-1} ($r \geq 0.995$), concentration 250 μg mL^{-1}: 300, 240, 180, 120, and 60 μg mL^{-1} ($r \geq 0.996$), and concentration 2 mg mL^{-1}: 2.20, 1.76, 1.32, 0.88, and 0.44 mg mL^{-1} ($r \geq 0.996$)].

HPLC

Equipment

Analyses were carried out using a Shimadzu HPLC (pumps: Shimadzu LC 10 AS; diode-array detector: Shimadzu SPD-M10A; column oven: Shimadzu CTO-10AC (20°C); rheodyne injection valve with a 20 μL loop). Separation was achieved on a MERCK LiChrospher® 100 RP 18 endcapped column 5 μm 119 mm long × 3 mm I.D.

Analytical Conditions

HPLC mobile phase was prepared using methanol–acetate buffer pH 4.6 (tetrabutylammoniumhydrogensulfate 0.0075 M). The mobile phase was filtered and degassed before use. Isocratic elution was employed with methanol–acetate buffer (pH 4.6, 0.4 M; tetrabutylammoniumhydrogensulfate 0.0075 M) (40 : 60 v/v).

Diode array detection was used, wavelengths set at absorption maximum of the substance ($\lambda = 280$ nm) and at 254 nm, the universal wavelength used for aromatic compounds. The peak purity index for the drug substance was investigated and found to be better than 0.9991 in chromatograms of the standard compounds, as well as in the chromatograms of the stressed solutions. For quantitation, external calibration was carried out. For each concentration range, five standard solutions were prepared, considering the dilution of the samples

before injection as well. Linear calibration functions resulted in all cases [concentration 40 μg mL^{-1}: according 44.0, 35.2, 26.4, 17.6, and 8.8 μg mL^{-1} ($r \geq 0.9986$), concentration 250 μg mL^{-1}: according 300, 240, 180, 120, and 60 μg mL^{-1} ($r \geq 0.9989$), and concentration 2 mg mL^{-1}: according 2.20, 1.76, 1.32, 0.88, and 0.44 mg mL^{-1} ($r \geq 0.9989$)].

CE

Equipment

Analyses were carried out using a Hewlett Packard ^{3D}CE equipped with an uncoated capillary (HP), 40 cm effective length, I.D. 50 μm.

Analytical Conditions

A new capillary was flushed consecutively with 1 M and 0.1 M sodium hydroxide (15 min each) and water (at least 30 min) before use. Before each injection, the capillary was preconditioned by flushing with run buffer for 5 min. Samples were injected by pressure of 50 mbar for 3.00 sec. For separation, a 20 mM sodium phosphate buffer solution pH 8.0 was used; a voltage of 25 kV was applied. The temperature of the capillary compartment was 25°C.

Diode array detection was used, wavelengths set at absorption maximum of the substance ($\lambda = 280$ nm) and at 254 nm, the universal wavelength used for aromatic compounds, and at 214 nm. The peak purity index for the drug substance was investigated and found to be better than 0.990 in electropherograms of the standard compounds, as well as in the electropherograms of the stressed solutions. For quantitation, external calibration was used. For each concentration range, five standard solutions were prepared. Linear calibration curves were obtained in all cases [concentration 40 μg mL^{-1}: 44.0, 35.2, 26.4, 17.6, and 8.8 μg mL^{-1} ($r \geq 0.996$), concentration 250 μg mL^{-1}: 300, 240, 180, 120, and 60 μg mL^{-1} ($r \geq 0.998$) and concentration 2 mg mL^{-1}: 2.20, 1.76, 1.32, 0.88, and 0.44 mg mL^{-1} ($r \geq 0.998$)].

Light Conditions

The sample solutions (10 mL each in a 10 mL volumetric flask) were exposed to forced irradiation using a Suntest CPS Accelerated Exposure Machine (Heraeus, Hanau, Germany; Art.No. 55007014): xenon burner NXE 1500, black panel temperature: 49°C, radiation intensity (1300 W/m^2); window glass filter (Art.No. 56009562) ; time factor: 15 (1 min Suntest ≅ 15 min bright sunlight). Distance of source to specimen table 22 cm.

RESULTS AND DISCUSSION

Three different analytical assays (HPTLC/densitometry, HPLC, and CE) were developed, allowing selective quantitation of tenoxicam in presence of its degradation products. A comparison of the results obtained with the three different analytical methods, seemed of interest. HPLC today is, and will be, the most frequently used method in drug analysis. In the future, CE in addition to HPLC will have an increasing importance, since it is a rapid, selective method, which requires little quantity of mostly aqueous buffers with a minimum of costs and problems for the environment. Results obtained with HPTLC/densitometry as a classic, still well suited method for stability testing in drug analysis, are compared with those obtained by the two other methods as well, since it seemed interesting to show a comparison between those three methods applied to the same problem to evaluate their usefulness for the photostability testing of tenoxicam.

The stability indicating capability of the assays was proved using sample solutions, subjected to forced degradation by exposing them to artificial irradiation from a xenon source in a Suntest. Different exposure times were chosen for the respective concentrations. The Suntest is an accelerated exposure machine rated at 15 times the intensity of sunlight, thus, leading to reduced testing time. It provides radiation distribution, as well as relative intensities at the different wavelengths, similar to natural sunlight and reproducible conditions giving repeatable levels of irradiation, which is not guaranteed when reliance is placed on varying intensities of natural sunlight.

The resulting chromatograms and the electropherogram of the stressed solution are shown in Figs. 1–3. In all cases, the degradation products are well resolved from the peak of tenoxicam. The different numbers of degradation products shown in the chromatograms and electropherogram, are due to the different detection limits and different detection wavelengths. The used CE method separated some of the degradation products, which co-eluted in HPLC. The separation of all degradation products was not necessary, since the study was exclusively aimed at the selective determination of tenoxicam. No degradation products overlap the peak of tenoxicam, which was proved by assessing the peak purity (comparison of remission spectra in HPTLC, peak purity index in HPLC in all cases found to be better than 0.9991, peak purity index in CE better than 0.990).

The methods were validated by evaluation of intra- and inter-day precision on the basis of quantitative results by external calibration for nine replicate injections. The relative standard deviations (RSDs) of the used HPTLC/densitometry method (Table 1) were found to be between 1.81% and 2.54% ($2\,mg\,mL^{-1}$), 2.23% and 2.67% ($250\,\mu g\,mL^{-1}$), and 1.80% and 3.02% ($40\,\mu g\,mL^{-1}$) in the intra-day assay. The RSD in the inter-day assay (3 days, $n = 9$) was 2.44% for $2\,mg\,mL^{-1}$, 2.60% for $250\,\mu g\,mL^{-1}$, and 2.59% for

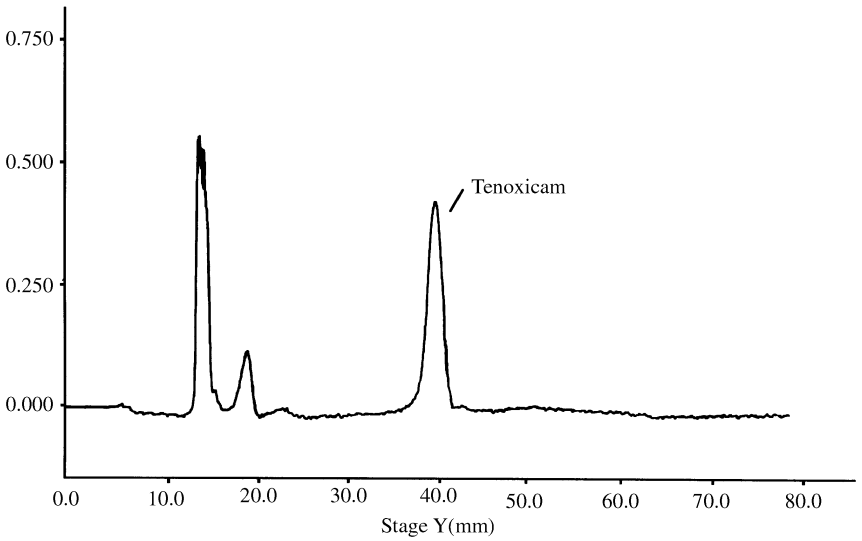

Figure 1. HPTLC chromatogram of tenoxicam (2 mg/mL) irradiated for 10 h in the Suntest.

40 μg mL^{-1}. The RSDs of the used HPLC method (Table 2) were found to be between 0.68% and 1.01% (2 mg mL^{-1}), 0.91% and 1.68% (250 μg mL^{-1}), and 0.78% and 1.47% (40 μg mL^{-1}) in the intra-day assay. The RSD in the inter-day assay (3 days, $n = 9$) was 2.17% for 2 mg mL^{-1}, 1.92% for 250 μg mL^{-1}, and 2.74% for 40 μg mL^{-1}. The RSDs of the used CE method (Table 3) were found to

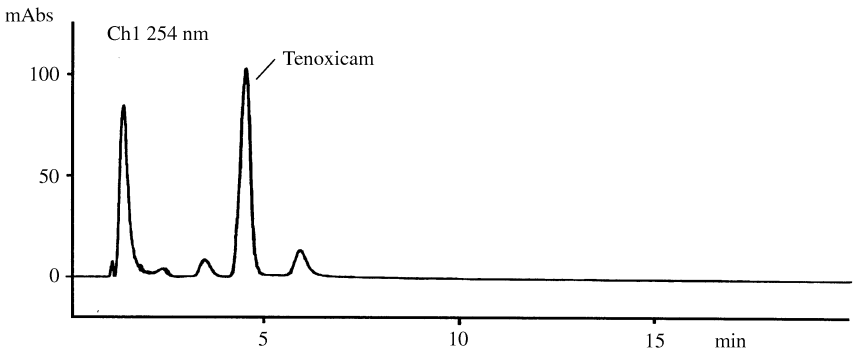

Figure 2. HPLC chromatogram of tenoxicam (40 μg/mL) irradiated for 48 min in the Suntest.

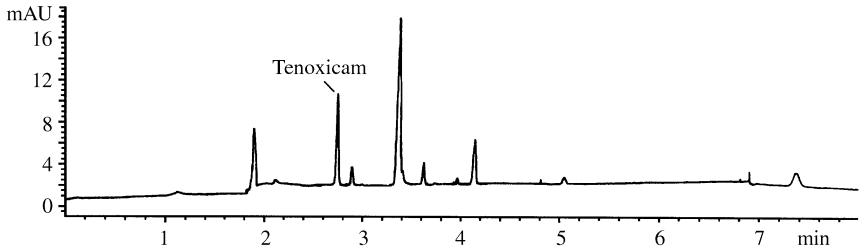

Figure 3. CE electropherogram of tenoxicam (250 μg/mL) irradiated for 96 min in the Suntest.

be between 1.48% and 2.85% (2 mg mL^{-1}), 1.98% and 3.50% (250 μg mL^{-1}), and 1.60% and 2.59% (40 μg mL^{-1}) in the intra-day assay. The RSD in the inter-day assay (3 days, $n = 9$) was 4.85% for 2 mg mL^{-1}, 3.35% for 250 μg mL^{-1}, and 4.52% for 40 μg mL^{-1}.

For the photostability testing, tenoxicam solutions of three different concentrations (2 mg mL^{-1}; 250 μg mL^{-1}; 40 μg mL^{-1}) were subjected to simulated sunlight for 192 min. Samples were removed at certain times and tested for the amount of tenoxicam remaining in the solution. Light exposure leads to degradation of different extents, strongly dependent on the respective concentration of the drug substance. The results of the three different analytical methods correspond very well, though surprisingly, the remaining concentration found via CE in all cases was a little less than that found with the other methods.

Table 1. Intra- and Inter-Day Precision of the Proposed HPTLC/Densitometry Assay

	Intra-Day Precision			Inter-Day Precision		
Conc. (μg mL^{-1})	n	Found Mean Conc. ± S.D. (μg mL^{-1})	RSD (%)	n	Found Mean Conc. ± S.D. (μg mL^{-1})	RSD (%)
2,000	9	1,915 ± 35	1.81			
2,000	9	1,839 ± 41	2.18	3	1,918 ± 47	2.44
2,000	9	1,948 ± 49	2.54			
250	9	250.2 ± 6.2	2.48			
250	9	246.1 ± 6.6	2.67	3	246.8 ± 6.4	2.60
250	9	244.0 ± 5.4	2.23			
40	9	41.7 ± 1.2	2.89			
40	9	41.7 ± 0.7	1.80	3	41.9 ± 1.1	2.59
40	9	42.2 ± 1.3	3.02			

Table 2. Intra- and Inter-Day Precision of the Proposed HPLC Assay

	Intra-Day Precision			Inter-Day Precision		
Conc. ($\mu g\,mL^{-1}$)	n	Found Mean Conc. ± S.D. ($\mu g\,mL^{-1}$)	RSD (%)	n	Found Mean Conc. ± S.D. ($\mu g\,mL^{-1}$)	RSD (%)
2,000	9	2,022 ± 16	0.81			
2,000	9	2,013 ± 20	1.01	3	1,990 ± 43	2.17
2,000	9	1,935 ± 13	0.68			
250	9	261.5 ± 2.4	0.91			
250	9	256.9 ± 4.3	1.68	3	261.6 ± 5.0	1.92
250	9	266.4 ± 2.6	0.99			
40	9	42.2 ± 0.6	1.47			
40	9	44.5 ± 0.5	1.15	3	43.7 ± 1.2	2.74
40	9	44.4 ± 0.3	0.78			

Explanation of these findings was brought by a closer look at the samples withdrawn from the irradiated solutions for analyses. The autosampler vials used in this study for the CE, needed to be filled with approximately 700 μL sample solution. Samples taken for the other methods were less than that. Since the stability of the tenoxicam solutions were found to be highly dependent of the concentration, (Fig. 4), a dependency of the volume of the solution exposed to irradiation could also be suspected. This assumption could be proven with

Table 3. Intra- and Inter-Day Precision of the Proposed CE Assay

	Intra-Day Precision			Inter-Day Precision		
Conc. ($\mu g\,mL^{-1}$)	n	Found Mean Conc. ± S.D. ($\mu g\,mL^{-1}$)	RSD (%)	n	Found Mean Conc. ± S.D. ($\mu g\,mL^{-1}$)	RSD (%)
2,000	9	1,968 ± 29	1.48			
2,000	9	1,899 ± 52	2.74	3	1,881 ± 91	4.85
2,000	9	1,777 ± 51	2.85			
250	9	230.6 ± 8.1	3.50			
250	9	241.5 ± 5.8	2.42	3	234.6 ± 7.86	3.35
250	9	231.8 ± 4.6	1.98			
40	9	39.7 ± 0.9	2.37			
40	9	43.3 ± 1.1	2.59	3	42.0 ± 1.9	4.52
40	9	43.0 ± 0.7	1.60			

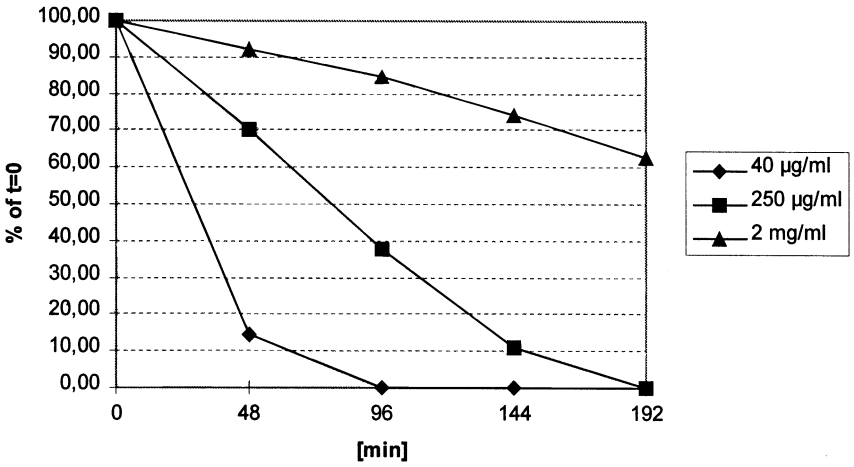

Figure 4. Degradation of solutions containing different concentrations of tenoxicam after irradiation in the Suntest.

samples of 10.0 mL containing tenoxicam 250 µg/mL, prepared and tested in triplicate; the quantitation was made by HPTLC and by CE, respectively. The solutions were irradiated in the Suntest and samples of defined volumes (200 µL, 700 µL, and 700 L, respectively) were taken at the very same sampling times as in the previous experiments. After 96 min, the sampling of 200 µL lead to an average of 46% of the initial concentration, whereas after sampling of 700 µL each, only 23% of the initial amount was found. No difference between the results of HPTLC and of CE were observed when the sampling volume was equal. This explains why care should be taken with interpretation of results of photostability studies, since without details of all experimental parameters a comparison of results is hardly reliable.

Investigations of samples exposed to natural daylight showed inhomogen results, due to varying light conditions between the different testing times. These findings prove the importance of reproducible light conditions to obtain reliable results. No hydrolytic degradation was observed with samples stored under light protection at elevated temperature (50°C).

The RSD for the three methods at each concentration (Tables 1–3) as could be expected, gave the best results for quantitation utilizing HPLC. HPTLC/densitometry and CE showed higher RSDs, especially at low sample concentrations. Since migration time reproducibility might lead to problems in the CE, a migration time validation was carried out ($n = 9$), the migration times found to be 2.780 ± 0.016 (RSD 0.57%). Detection and quantitation limits are lowest with HPLC; the results for the three used methods are given in Table 4.

Table 4. Comparison of Detection and Quantitation Limits

Method	LOD	LOQ
HPTLC	2 μg/mL	7.5 μg/mL
HPLC	0.20 μg/mL	0.75 μg/mL
CE	1 μg/mL	5 μg/mL

CONCLUSION

The photostability of tenoxicam was tested, exposing sample solutions to irradiation from a xenon source. The photodegradation of tenoxicam was found to be highly dependent on the concentration and the volume of the sample solution. All three assay methods proposed for selective quantitation of tenoxicam in presence of its degradation products, proved to be well suited. The comparison of the results obtained by the employed CE and HPLC assay, was of special interest, since generally, stability tests utilizing CE have not been frequently reported yet. The importance of using an artificial sunlight simulation was emphasized by the results obtained with solutions exposed to natural daylight, the latter results showing higher deviation according to varying light intensities.

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Received March 31, 2002
Accepted May 10, 2002
Manuscript 5838