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Stability indicating assays for the determination of piroxicam—comparison of methods☆

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Abstract

Photodegradation of piroxicam, a 1,2-benzothiazine oxicam, is studied laying special emphasis on the investigation of the correlation between concentration of the sample solution and stability. A comparison of three different methods (HPTLC/densitometry, HPLC, CE) developed for the photostability testing of the title compound is presented. The stability indicating capability of the assays is proved using forced degradation by exposing a sample solution to artificial irradiation from a xenon source. The chromatograms and the electropherogram of the resulting solution show piroxicam well resolved from the degradation products. For quantitation external calibration is employed, all calibration curves being linear in the respective concentration range of interest. Piroxicam solutions of three different concentrations (2 mg ml^{-1}; 250 μg ml^{-1}; 40 μg ml^{-1}) are subjected to simulated sunlight for 480 min. The stability is investigated by quantitation of piroxicam by the methods mentioned. The methods are compared in respect of performance and precision. Costs and time of analysis are regarded also. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Piroxicam; Antirheumatic drug; Photostability; Stability indicating assays; TLC; HPLC; CE

1. Introduction

Piroxicam, a 1,2-benzothiazine oxicam, is a potent anti-inflammatory and antirheumatic drug. The structure comprises two possible tautomeric forms (Fig. 1). The drug is quite efficient in the treatment of rheumatoid arthritis, osteoarthrosis, ankylosing spondylitis and acute pain in musculoskeletal disorders and acute gout [1] and has a long half-life. It has been reported to possibly show phototoxic potential [2] which might be caused by photodegradation products. Stability of piroxicam previously has been studied in aqueous media as a function of pH but without considering light influence [3]. Chromatographic systems to separate piroxicam from three main degradation products and precursors of synthesis, 2-

☆ Dedicated to Professor Gottfried Heinisch (University of Innsbruck, Austria) with best wishes on the occasion of his 60th birthday.

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aminopyridine, 2-methyl-2H-1,2-benzothiazin-4(3H)-one 1,1-dioxide and *N*-methyl-*N*′-(2-pyridinyl)-ethane-diamide have been proposed [4]. Contradictory data concerning the photostability of the oxicams can be found in the literature. Piroxicam solutions (1% in aqueous media) exposed to sunlight were found to be stable for 72 h [5]. On the other hand aqueous solutions (~ 2.3 μg ml^{-1}) of tenoxicam, a thienothiazine oxicam derivative, showed ~ 50% loss of the drug when exposed to sunlight for 3 h [6].

These diverse results promoted an interest to carry out a comprehensive study on the photostability of the oxicams. Special emphasis is laid on different factors influencing the extent and the rate of photodegradation of the compounds since this has not been taken into account in the previously published investigations. Our study which is currently under investigation is considering the influence of different light sources and different sample concentrations. We found the stability of tenoxicam to be dependent of the nature of light, a marked concentration dependency was observed as well [7]. This concentration dependency of the photodegradation of the oxicams might explain the different results published [5,6], and points out the importance to consider manifold factors influencing the photostability of drugs. In this paper a study on the stability of piroxicam is presented. 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 piroxicam. A comparison of three different stability indicating methods (HPTLC/densitometry, HPLC, CE) developed for the photostability testing of piroxicam in simulated sunlight is included as well. The stability was investigated by quantitation of piroxicam by the methods mentioned. The methods are compared in respect of performance and precision. Costs and time of analysis are regarded as well.

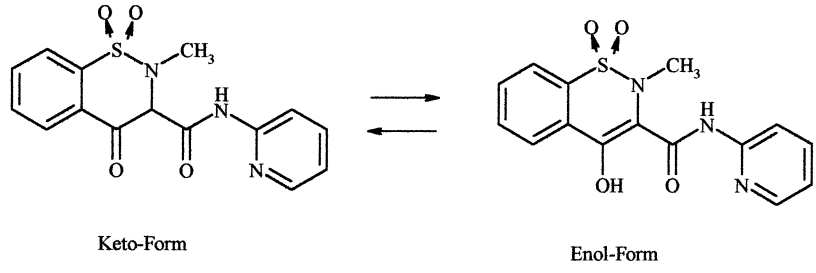

Fig. 1. Tautomeric forms of piroxicam.

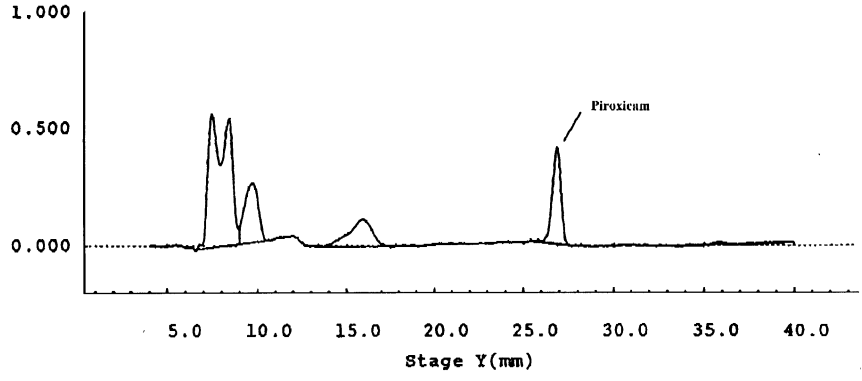

Fig. 2. HPTLC chromatogram of piroxicam (2 mg ml^{-1}) irradiated for 18 h in the Suntest.

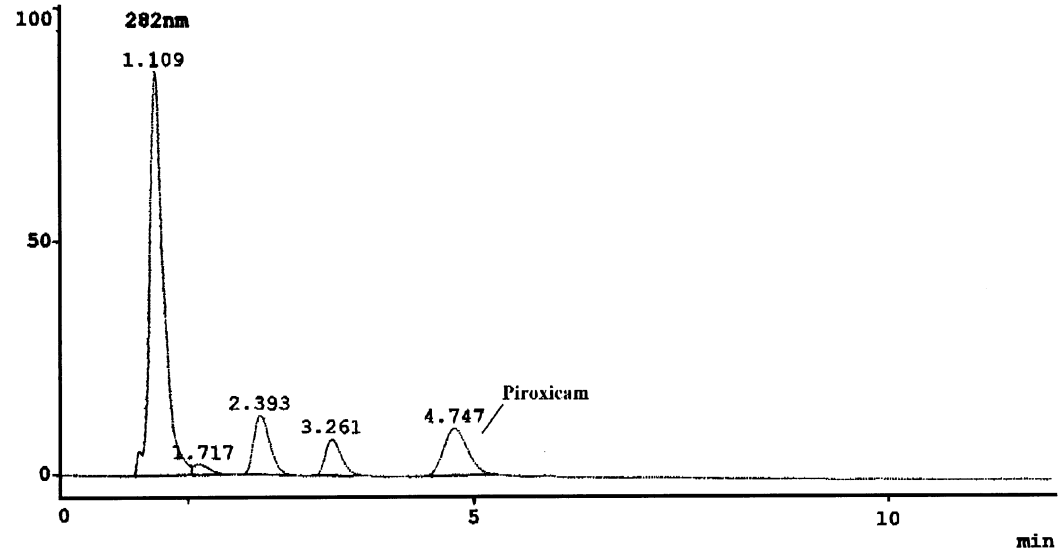

Fig. 3. HPLC chromatogram of piroxicam (40 μg ml^{-1}) irradiated for 2 h in the Suntest.

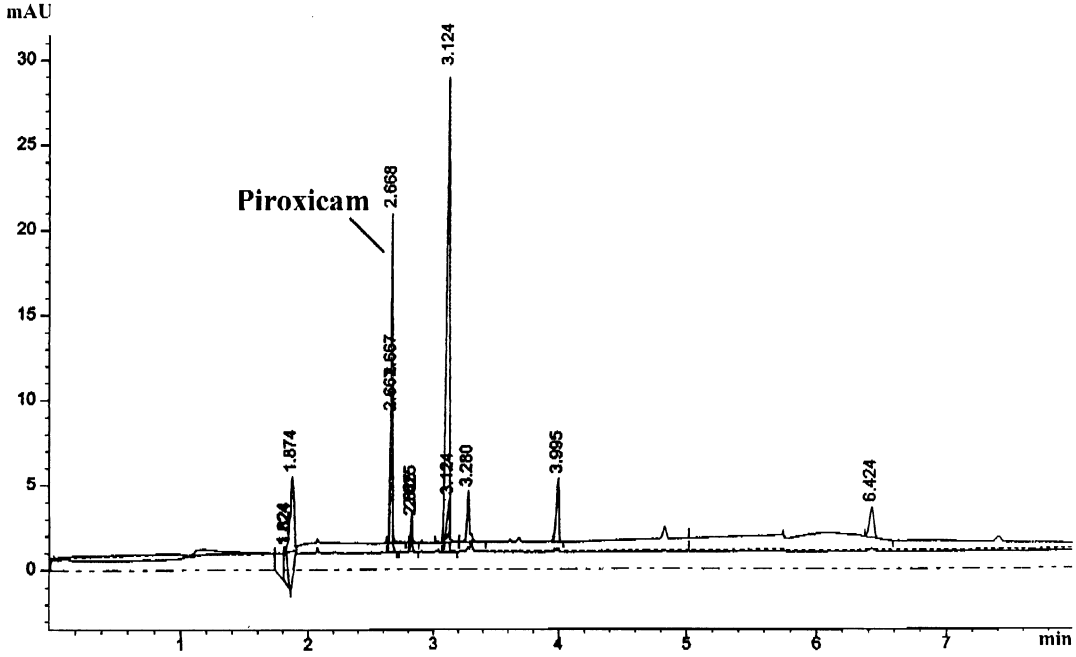

Fig. 4. CE electropherogram of piroxicam (250 μg ml^{-1}) irradiated for 6 h in the Suntest.

2. Experimental

2.1. Materials

The drug substance was obtained from Sigma (Vienna, Austria) and stored at 2–8°C. 25% NH_4OH solution analytical grade was obtained from Riedel-de Haen (Seelze, Germany). For HPTLC chloroform, methanol 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 4.3 were of analytical grade. 20 mM sodium phosphate buffer solution pH 8.0 for HPCE was obtained from Fluka Chemie AG (Buchs, Switzerland).

2.2. Sample preparation

Solutions containing piroxicam 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).

2.3. 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 chromato-

Table 1
Intra- and interday precision of proposed HPTLC/densitometry method

Intraday precision				Interday precision		
Conc. (μg ml^{-1})	*n*	Found mean conc. ± SD (μg ml^{-1})	RSD (%)	*n*	Found mean conc. ± SD (μg ml^{-1})	RSD (%)
2000	9	1905.7 ± 28.57	1.50	3	1925.6 ± 46.36	2.41
2000	9	1899.8 ± 33.39	1.76			
2000	9	1971.4 ± 38.62	1.96			
250	9	251.4 ± 5.58	2.22	3	249.6 ± 5.94	2.38
250	9	249.7 ± 4.64	1.86			
250	9	250.7 ± 5.54	2.21			
40	9	42.3 ± 1.36	3.22	3	41.3 ± 1.45	3.50
40	9	41.1 ± 1.21	2.95			
40	9	40.6 ± 1.35	3.32			

Table 2
Intra- and interday precision of proposed CE method

	Intraday precision			Interday precision		
Conc. (μg ml^{-1})	*n*	Found mean conc. ± SD (μg ml^{-1})	RSD (%)	*n*	Found mean concentration ± SD (μg ml^{-1})	RSD (%)
2000	9	1933.6 ± 33.62	1.74	3	2033.4 ± 88.43	4.35
2000	9	2065.7 ± 66.29	3.21			
2000	9	2101.0 ± 48.60	2.31			
250	9	255.1 ± 8.93	3.50	3	251.4 ± 9.40	3.74
250	9	243.7 ± 6.72	2.76			
250	9	255.3 ± 7.90	3.10			
40	9	41.3 ± 1.49	3.61	3	42.2 ± 1.62	3.84
40	9	43.7 ± 1.08	2.47			
40	9	41.7 ± 1.29	3.09			

Table 3
Intra- and interday precision of proposed CE method

Intraday precision				Interday precision		
Conc. (μg ml^{-1})	n	Found mean conc. ± SD (μg ml^{-1})	RSD (%)	n	Found mean conc. ± SD (μg ml^{-1})	RSD (%)
2000	9	1933.6 ± 33.62	1.74	3	2066.8 ± 48.35	2.34
2000	9	2065.7 ± 66.29	3.21			
2000	9	2101.0 ± 48.60	1.35			
250	9	263.4 ± 2.34	0.89	3	263.8 ± 5.85	2.22
250	9	270.2 ± 3.01	1.11			
250	9	257.9 ± 3.42	1.33			
40	9	43.9 ± 0.47	1.07	3	42.9 ± 1.21	2.83
40	9	41.3 ± 0.50	1.22			
40	9	43.5 ± 0.37	0.86			

Table 4
Degradation of 1 in solutions (% of initial concentration)[a]

Time (min)	2 mg ml^{-1}			250 μg ml^{-1}			40 μg ml^{-1}		
	HPTLC	HPLC	CE	HPTLC	HPLC	CE	HPTLC	HPLC	CE
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
24	99.97	97.85	97.02	97.33	97.90	91.12	86.60	84.71	87.77
48	94.68	98.53	97.40	90.74	92.99	87.42	75.12	71.50	66.43
96	92.69	97.85	96.65	85.34	82.72	79.23	49.24	41.69	33.67
144	92.28	94.86	93.66	76.46	70.82	64.65	28.92	18.62	15.23
192	90.83	93.25	91.29	64.82	61.01	52.51	14.13	7.82	[b]
288	88.19	88.34	82.76	51.90	35.84	34.42	0.49	0.70	[b]
384	80.18	82.50	75.55	21.26	17.71	14.64	[b]	[b]	[b]
480	76.13	75.96	66.83	1.14	3.63	2.39	[b]	[b]	[b]

graphic plates. Plates are prewashed before use with methanol/dichloromethane 1:1 v/v. 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 μg ml^{-1}). HPTLC mobile phase was chloroform–96% acetic acid (9/1 v/v).

The densitometric measurements were made at $\lambda = 280$ nm using a 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 piroxicam peak of freshly prepared as well as stressed solutions proved that no degradation products overlap the peak of piroxicam. For quantitation, external calibration was carried out. For each concentration range, five standard solutions were prepared. Linear calibration curves were obtained 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}, respectively ($r \geq 0.996$), concentration 250 μg ml^{-1}: 300, 240, 180, 120 and 60 μg ml^{-1}, respectively ($r \geq 0.997$) and concentration 2 mg ml^{-1}: 2.20, 1.76, 1.32, 0.88 and 0.44 mg ml^{-1}, respectively ($r \geq 0.997$)).

2.4. HPLC

Equipment: Analyses were carried out using a Shimadzu HPLC (pumps: SHIMADZU LC 10 AS; diode-array detector: SHIMADZU

Table 5
Degradation of 1 in solutions (% of initial concentration)[a]

Time (h)	2 mg ml^{-1}			250 μg ml^{-1}			40 μg ml^{-1}		
	HPTLC ($n=9$)	HPLC ($n=3$)	CE ($n=3$)	HPTLC ($n=9$)	HPLC ($n=3$)	CE ($n=3$)	HPTLC ($n=9$)	HPLC ($n=3$)	CE ($n=3$)
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
6	100.11	99.44	97.43	103.06	99.61	102.06	99.31	98.14	101.68
12	98.55	99.38	96.40	100.79	98.35	98.94	94.50	98.40	100.48
24	98.97	98.15	92.15	100.03	99.97	96.63	92.25	98.46	94.89
36	99.02	100.51	93.91	95.80	100.44	93.04	89.25	100.25	85.63
48	98.22	99.45	89.77	94.48	98.45	89.93	87.88	101.26	65.84
72	99.96	97.42	92.89	95.28	99.85	85.98	85.05	97.67	50.84
96	99.68	97.95	91.60	94.03	96.75	82.56	84.62	92.43	43.66
120	98.65	96.80	91.77	90.49	97.90	84.67	77.01	93.32	34.40

[a] (100% at $t=0$). Exposure to daylight.

Table 6
Degradation of 1 in solutions (% of initial concentration)[a]

Time (min)	2 mg ml^{-1}			250 μg ml^{-1}			40 μg ml^{-1}		
	HPTLC ($n=9$)	HPLC ($n=2$)	CE ($n=3$)	HPTLC ($n=9$)	HPLC ($n=2$)	CE ($n=3$)	HPTLC ($n=9$)	HPLC ($n=2$)	CE ($n=3$)
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
24	98.38	100.98	99.33	101.61	99.56	95.54	98.87	98.11	101.89
48	95.32	97.69	101.09	104.28	101.81	96.69	99.40	99.66	100.82
96	94.56	100.41	98.89	102.81	99.81	97.20	97.74	100.20	99.92
144	94.52	99.91	100.75	103.07	99.98	96.82	100.32	100.31	101.48
192	101.89	98.27	101.05	98.19	101.72	99.93	99.72	99.47	101.64
288	97.48	100.43	102.84	95.53	100.97	97.64	97.94	97.29	99.34
384	94.98	102.15	100.90	98.50	101.65	99.77	97.44	100.96	100.00
480	94.54	99.85	103.96	98.56	101.53	96.66	97.04	98.87	99.67

[a] (100% at $t=0$). Storage under light protection (50°C).

SPD-M10A; column oven: SHIMADZU CTO-10AC (20°C); rheodyne injection valve with a 20 μl loop). Separation was achieved on a MERCK column LiChrospher® 100 RP 18 endcapped 5 μm 119 mm long × 3 mm I.D.

Analytical conditions: HPLC mobile phase was prepared using methanol–acetate buffer pH 4.3. The mobile phase was filtered and degassed before use. Isocratic elution was employed with methanol–acetate buffer (pH 4.3, 0.4 M) (45:55 v/v).

Diode array detection used wavelengths set at an 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 0.9997 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 also. 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}, respectively ($r \geq 0.9995$), concentration 250 μg ml^{-1}: 300, 240, 180, 120 and 60 μg ml^{-1}, respectively ($r \geq 0.9995$) and concentration 2 mg ml^{-1}: 2.20, 1.76, 1.32, 0.88 and 0.44 mg ml^{-1}, respectively ($r \geq 0.9989$)).

2.5. 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 with 1 and 0.1 M sodium hydroxid (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 applying a pressure of 50 mbar for 3 s. For separation, a 20 mM sodium phosphate buffer solution pH 8.0 was used, and a voltage of 25 kV was applied at a temperature of 25°C.

Diode array detection used wavelengths set at an absorption maximum of the substance ($\lambda = 280$ nm) and at 254 nm, the universal wavelength used

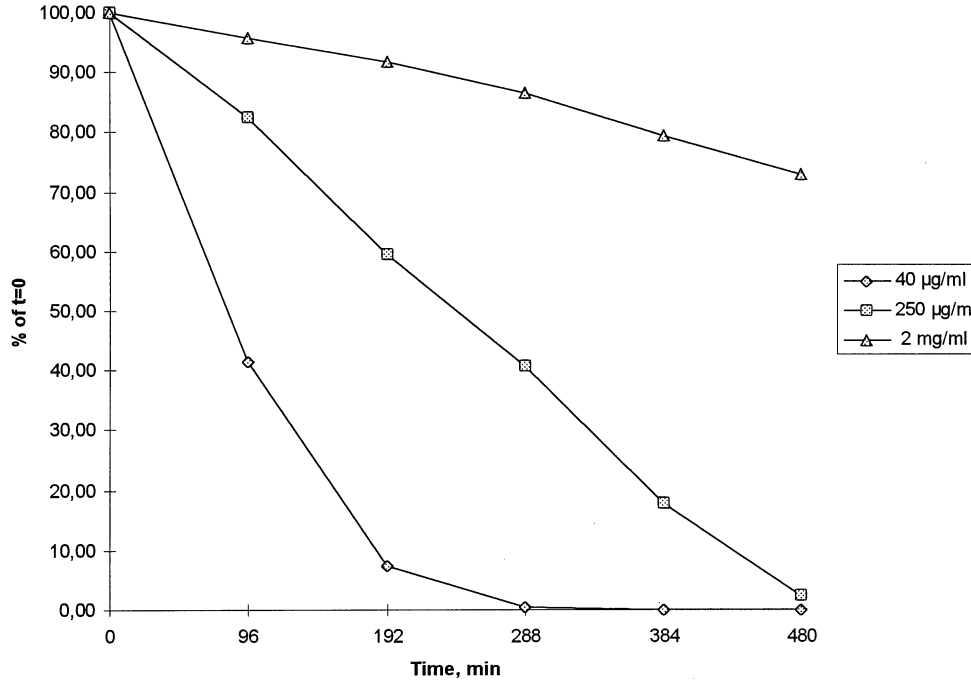

Fig. 5. Degradation of solutions containing different concentrations of piroxicam.

Table 7
Comparison of time of analysis and costs of the used methods

Method	Time and costs		
	Time for one analysis (min)	Costs of the instruments (Euro)	Costs for one analysis (Euro)
HPTLC	~30	~29 000	~1.08
HPLC	~15	~29 000	~0.72
CE	~15	~58 000	~0.18

for aromatic compounds and at 214 nm. The peak purity index for the drug substance was investigated and found to be better than 0.998 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}, respectively ($r \geq 0.996$), concentration 250 μg ml^{-1}: 300, 240, 180, 120 and 60 μg ml^{-1}, respectively ($r \geq 0.997$) and concentration 2 mg ml^{-1}: 2.20, 1.76, 1.32, 0.88 and 0.44 mg ml^{-1}, respectively ($r \geq 0.997$)).

2.6. 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 (1940 W m^{-2}); windowglass filter (Art. No. 56009562); time factor: 15 (1 min Suntest ≅ 15 min bright sunlight). Distance of source to specimen table, 22 cm.

3. Results and discussion

Three different analytical assays (HPTLC/densitometry, HPLC and CE) were developed aimed at the selective quantitation of piroxicam in the presence of its degradation products since none of the published separation systems [4] seemed to be suitable for this investigation. 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 a repeatable level of irradiation which is not guaranteed when reliance is placed on varying intensities of natural sunlight.

HPLC, which is and will in the future be the leading method in drug analysis, is employed. The resulting data should serve as a basis to compare those obtained by HPTLC and CE, respectively. This seemed interesting since HPTLC though being a classic method is still widely used especially in those laboratories which are only occasionally performing analytical investigations (e.g. laboratories of hospital pharmacies). TLC is an easy to use robust method and needs less technical know-how and less machine maintenance than HPLC, which still makes it a valuable method. In the beginning, CE was mostly used for the analysis of biomolecules as proteins and nucleic acids, but recently it is more and more applied as a method for drug analysis. Therefore, it seems interesting to show a comparison between those methods applied to the same problem to evaluate their usefulness for this photostability testing.

The resulting chromatograms and the electropherogram of the stressed solution are shown in Figs. 2–4. In all cases, the degradation products

are well resolved from the peak of piroxicam. The different number of degradation products shown in the chromatograms and electropherogram are due to the different detection limits and wavelengths. The CE method used separated some of the degradation products which co-eluted in the HPLC. However, since the aim of the study was mainly selective determination of piroxicam, no separation of all degradation products was necessary. No degradation products overlap the peak of piroxicam 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 0.9997, peak purity index in CE better than 0.998).

The methods were validated by the evaluation of intra- and inter-day precision. The relative standard deviations (RSD) of the used HPTLC/densitometry method (Table 1) on the basis of peak area ratios for nine replicate injections were found to be between 1.50 and 1.96% (2 mg ml^{-1}), 1.86 and 2.22% (250 μg ml^{-1}) and 2.95 and 3.32% (40 μg ml^{-1}) in the intra-day assay. The RSD in the inter-day assay (3 days, $n=9$) was 2.41% for 2 mg ml^{-1}, 2.38% for 250 μg ml^{-1} and 3.5% for 40 μg ml^{-1}. The RSD of the used HPLC method (Table 2) on the basis of quantitative results by external calibration for nine replicate injections were found to be between 0.91 and 1.35% (2 mg ml^{-1}), 0.89 and 1.33% (250 μg ml^{-1}) and 0.86 and 1.22% (40 μg ml^{-1}) in the intra-day assay. The RSD in the inter-day assay (3 days, $n=9$) was 2.34% for 2 mg ml^{-1}, 2.22% for 250 μg ml^{-1} and 2.83% for 40 μg ml^{-1}. The RSD of the used CE method (Table 3) on the basis of quantitative results by external calibration for nine replicate injections were found to be between 1.74 and 3.21% (2 mg ml^{-1}), 2.76 and 3.5% (250 μg ml^{-1}) and 2.47 and 3.61% (40 μg ml^{-1}) in the intra-day assay. The RSD in the inter-day assay (3 days, $n=9$) was 4.35% for 2 mg ml^{-1}, 3.74% for 250 μg ml^{-1} and 3.84% for 40 μg ml^{-1}.

For the photostability testing, piroxicam solutions of three different concentrations (2 mg ml^{-1}; 250 μg ml^{-1}; 40 μg ml^{-1}) were subjected to simulated sunlight for 480 min. Samples were removed at certain times and tested for the amount of piroxicam remaining in the solution. The results are given in Table 4. Light exposure leads to degradation of different levels depending on the respective concentration of the drug substance. The results of the three different analytical methods correspond very well. The concentration dependency of the photodegradation is distinctly shown (Fig. 5). Investigations of samples exposed to natural daylight showed varying results (compare Table 5) according to varying light conditions. Therefore, it can be emphasised that reproducible light conditions are important for obtaining reliable results. No hydrolytic degradation was observed with samples stored under light protection at elevated temperature (50°C) (Table 6).

As expected, the RSD for the three methods at each concentration (Tables 1–3) gave the best results for quantitation utilizing HPLC. HPTLC/densitometry and CE showed a higher standard deviation (SD) especially at low sample concentrations. Since migration time reproducibility might lead to problems in the CE, a migration time validation was carried out ($n=14$), the migration times were found to be 2.868 ± 0.014 (RSD 0.47%). A comparison of the time of analysis of each method showed densitometry to be most time consuming especially taking into account that it is not a fully automated method (Table 7). Even though CE-equipment is quite expensive, very low subsequent costs can be expected (Table 7). The costs calculated for one analysis include Hamilton syringe, HPTLC-plates and solvent costs for HPTLC. For HPLC, the costs for the Hamilton syringe, HPLC cartridge, filters for the aqueous mobile phase and the solvents are included. The costs for CE include vials, the capillary and the buffer.

4. Conclusion

The three assay methods proposed for selective quantitation of piroxicam in the presence of its degradation products proved to be suitable. The comparison of the results obtained by the employed CE and HPLC methods were of special interest since generally stability tests utilizing CE

have not been frequently reported up to now. The photostability of piroxicam in solution was tested using irradiation with a xenon source which yields light corresponding to natural sunlight with regard to wavelength distribution and relative intensities at the respective wavelengths. The importance of using an artificial sunlight simulation was pointed out by the results obtained with solutions exposed to natural daylight, the latter results showing higher deviation according to varying light intensity. The photodegradation of piroxicam was found to be highly dependent on the concentration of the sample solution which is proved by the quantitative results. Further investigations will be undertaken to elucidate the structure of the degradation products.

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