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A VALIDATED HPLC-ASSAY FOR THE DETERMINATION OF MELOXICAM IN PRESENCE OF ITS DEGRADATION PRODUCTS

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Abstract

The stability of aqueous solutions of meloxicam is studied with samples of different concentrations, and in different containers. Quantitation is carried out utilizing a validated stability indicating HPLC assay with five-point calibration. Sample solutions of meloxicam of three different concentrations (2 mg ml^{-1}; 250 µg ml^{-1}; 40 µg ml^{-1}) are subjected to simulated sunlight and tested for stability. A distinct correlation of the photodegradation rate with the concentration of the sample solution was found. Furthermore, the influence of size and geometry of the containers in which the solutions were exposed to light was investigated and results compared.

Keywords

meloxicam, HPLC, artificial sunlight, photostability

Introduction

Meloxicam is a non-steroidal antirheumatic of the oxicam-type with COX-2-inhibitory activity. The long biological half-life allows a once-daily administration, it is a potent antiinflammatory agent with less adverse effects as observed with other standard NSAID [1,2].

Few studies dealing with the determination of meloxicam by HPLC were published aimed mainly at the determination in bulk drug and drug preparations

[e.g. 3,4]. Meloxicam was analyzed by HPLC in presence of its alkaline degradation product utilizing a ternary eluent [5], spectrophotometric assays for this purpose have been published as well [6,7]. A validated stability indicating HPTLC assay with densitometric quantification has been proposed [8]. Previous studies proved a photoinstability for all oxicams under investigation, the rate of the photodegradation was dependent not only on the structure of the drug but also on various additional factors, especially the concentration of the tested sample solutions [8-10]. The current study should provide a validated HPLC assay to cross-validate the HPTLC assay [8] and elucidate the influence of different containers used for the light exposure of the samples on the stability of the respective sample solutions.

Results and Discussion

A previous study on the photostability of meloxicam [8] was based on a stability indicating HPTLC assay with densitometric quantitation. The sample solutions were exposed to simulated sunlight using volumetric flasks for containers. A significant degradation of the drug was observed, the extent being dependent on the concentration of the sample solution. Solutions stored under elevated temperatures but protected from light were stable during the test period, thus indicating that degradation is mainly caused by the light influence and not by the alkaline media used to solve the sample and the warming of the solution in the irradiation machine [8]. In continuation of this project, a HPLC assay is now presented, allowing selective determination of meloxicam in presence of the degradation products. A cross evaluation of the HPLC assay with the densitometric results was possible by the results of stability tests of drug solutions of equal concentrations as in the previous study. Antirheumatics of the oxicam type are well known to degrade upon light exposure, and some compounds of this group have been already investigated in this respect [e.g. 9,10]. In all cases, the degradation products have been of higher polarity than the drug. HPLC with an eluent containing methanol and acetate buffer of pH 4.6 40/60 v/v had been well suited for assaying the stability of isoxicam [9].

Consequently, this mobile phase was tested for suitability for the analysis of meloxicam as well. Good separation resulted, nevertheless due to the higher lipophilicity of meloxicam it was found to be advantagous to increase the ratio to 50% methanol in order to reduce the retention time. Selectivity of the assay was proved using a sample solution containing 250 µg/ml meloxicam. The sample was analyzed by HPLC immediately after preparation and after 864 minutes irradiation in the suntest. Meloxicam elutes at 4.14 minutes, the forcedly degraded solution showed several degradation products with retention times under 2 minutes (compare Fig. 1, 2) due to their higher polarity. No peak overlapped the peak of the analyte, the peak homogeneity was tested by the peak purity index which in all cases was found to be >0.999.

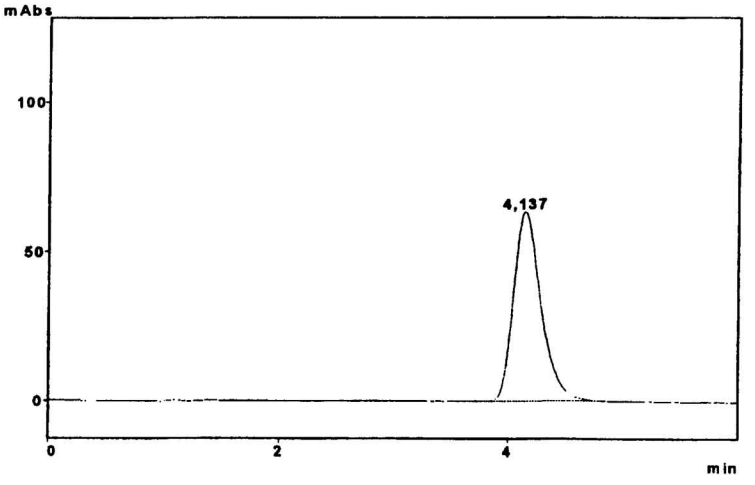

Fig. 1: Chromatogram of a freshly prepared solution of meloxicam (250 µg/ml)

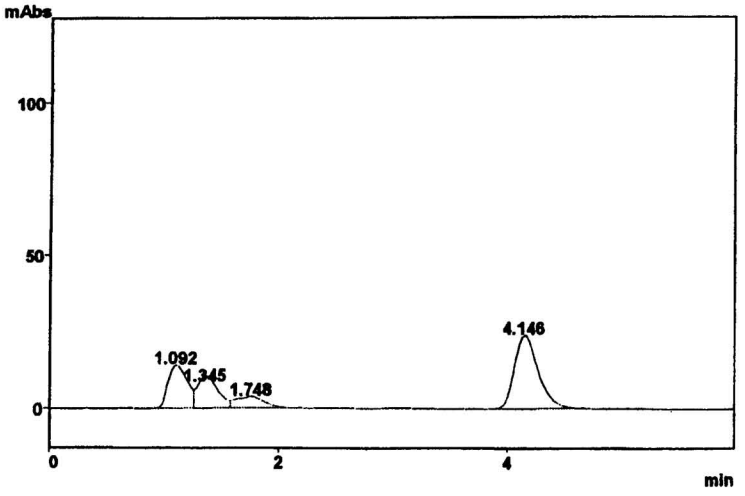

Fig. 2: Chromatogram of a solution of meloxicam (250 µg/ml) after 864 min irradiation in the Suntest

Satisfactory results have been obtained for the intra- and inter-day precision (see Tab. 1). The limit of detection (LOD; S/N 3:1) was 0.13 µg/ml, and the limit of quantitation (LOQ; S/N 10:1) was 0.25 µg/ml.

Conc.[µg/ml]	Intra-day (n=6) R.S.D. (%)	Inter-day (n=18) R.S.D. (%)
50	0.22-0.36	0.70
25	0.41-0.53	0.62
10	0.50-0.87	0.69
5	0.46-0.77	0.75
1	0.97-1.82	2.49
0.25	2.64-3.49	4.07

Tab. 1: Intra- and Inter-day Precision

The calibration functions were based on five standard concentrations covering the range of the respective sample concentrations (see Experimental). All functions were found to be linear, correlation coefficients were always better than 0.998.

The results for the stability tests of meloxicam in aqueous solutions of three different concentrations are given in Table 2. The data correlate very well with the data obtained by the TLC assay [8].

t [min]	2 mg/ml		250 µg /ml		40 µg /ml	
	conc. [µg/ml]	% of t=0	conc. [µg/ml]	% of t=0	conc. [µg/ml]	% of t=0
0	1992.31	100.0	249.52	100.0	40.52	100.0
144	1903.80	95.6	230.34	92.4	28.47	70.3
288	1869.15	93.8	209.05	83.8	13.93	34.4
432	1886.90	94.7	183.20	73.4	6.81	16.8
576	1836.53	92.2	162.15	65.0	3.73	9.2
720	1762.20	88.5	135.69	54.4	---	---
864	1737.49	87.2	109.32	43.8	---	---

--- = not detectable

Tab. 2: Concentration of meloxicam in solutions after irradiation in the Suntest (average, n=9); Container: 10 ml volumetric flask

Additionally, the photostability of meloxicam was now studied in different containers to investigate the influence of the geometry and the size of the container. This is of particular interest especially when drugs are parenterally applied as infusions, where mostly no special care is taken to protect the infusion bottles from ambient light or sunlight. Consequently, the stability tests were not only carried out in laboratory volumetric flasks but in infusion bottles of two different sizes as well. The results are given in Table 3. Based on equal initial sample volumes, the degradation was highest in a 250 ml infusion bottle, due to the thinner glass and to the greater surface area exposed to the irradiation. The sampling volume was 500 µl for each sampling step thus reducing the initial volume of 10 ml significantly after taking several aliquots. Further studies varying the sampling volume (200 and 700 µl, respectively) have been undertaken, but indicated no significant influence on the

remaining amount of meloxicam. However, it can be recommended that the volume exposed to the irradiation as well as the volume of the sampling should to be specified exactly in order to guarantee good reproducibility of the results.

	% of initial concentration		
t [min]	10 ml volumetric flask	100 ml infusion bottle	250 ml infusion bottle
0	100.0	100.0	100.0
144	92.4	88.7	79.8
288	83.8	80.1	72.1
432	73.4	68.2	57.3
576	65.0	62.6	47.1
720	54.4	47.4	32.8
864	43.8	36.0	20.8

Tab. 3: Stability of meloxicam in solutions stored in different containers / Irradiation in the Suntest (average, n=9); Initial Concentration 250 µg/ml, Initial Volume 10.00 ml

Experimental

Meloxicam was donated by Boehringer-Ingelheim, Vienna. Simulated Sunlight: exposition in the Suntest CPS Heraeus Accelerated Exposure Machine; Xenon-light source NXE 1500; distance source - specimen table: 22 cm, black panel temperature: 49°C at maximum intensity (~1300 W/m^2); window glas filter; time factor: 15 (4 min Suntest ≅ 1 h natural sunlight); Luxmeter: XenoCal/XenoSoft. Oven: Heraeus Instruments, temperature 50° ± 1° C. HPLC equipment: pumps Shimadzu LC 10AS, column oven Shimadzu CTO 10AC, diode-array detector Shimadzu SPD-M10A, autosampler Shimadzu SIL-10ADVP. Column: EcoCART® 125-3 LiChrospher® 100 RP-18 endcapped, 119x3mm, particle size 5µm. Mobile

Phase: methanol - acetate buffer pH 4.6 (50/50 v/v) (methanol and water 'Baker HPLC analyzed' by J.T.Baker, sodium acetate trihydrate and acetic acid 96% analytical grade by Merck); the mobile phase was filtered and degassed before use. Flow rate: 0.8 ml/min; Column temperature 20°C; Detection: λ=272 nm

Calibration: Five point external calibrations were used for quantitation of the samples. For the samples of 40 µg/ml standard concentrations were 44, 35.2, 26.4, 17.6 and 8.8 µg/ml, respectively. The standard solutions were diluted 1:1 with eluent before injection. For the samples of 250 µg/ml standard concentrations were 300, 240, 180, 120 and 60 µg/ml, respectively. The standard solutions were diluted 1:10 with eluent before injection. For the samples of 2 mg/ml standard concentrations were 2200, 1760, 1320, 880 and 440 µg/ml, respectively. The standard solutions were diluted 1:50 with eluent before injection. All solutions are subjected to the HPLC immediately after dilution with the eluent.

Validation: The validation was conducted with six concentrations (50, 25, 10, 5, 1 and 0.25 µg/ml, respectively). Each concentration was analyzed six times each on three different days to determine the intra-day and the inter-day precision. Selectivity proof was carried out using a sample solution of 250 µg/ml which was subjected to irradiation in the Suntest for 864 minutes. For the determination of the LOD and the LOQ sample solutions were diluted until the S/N ratio was 10:1 for the LOQ and 3:1 for the LOD.

Test conditions: Solutions of meloxicam in 2.5% aqueous ammonia (pH 12.57) of concentrations of 40 µg/ml; 250 µg/ml and 2 mg/ml, respectively, were exposed to irradiation in the Suntest, samples of 500µl were taken at t= 0, 144, 288, 432, 576, 720 und 864 min, three sample solutions were prepared of each concentration and stored in the respective container; these experiments were repeated two times, the average results (n=9) are given in Table 2. Irradiation tests were done placing the sample solutions in different containers: volumetric flasks of white glass, 10 ml ±0.04 Duran NS 10/19; infusion bottles 100 ml ISO II 18; and infusion bottles 250 ml ISO II SGD. Before injection in the HPLC the sample solutions were diluted with the

eluent mixture methanol/acetate buffer pH 4.6 1:1 (samples of 40 µg/ml were diluted 1:1, samples of 250 µg/ml were diluted 1:10, samples of 2 mg/ml were diluted 1:50). All solutions were subjected to the HPLC immediately after dilution with the eluent.

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