Stability-Indicating High-Performance Liquid Chromatographic Assay Method and Photostability of Carprofen

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Abstract

A rapid, sensitive, and accurate stability-indicating high-performance liquid chromatographic assay method for determining the degradation of carprofen (CPF) is developed and validated under acidic, basic, or photo-irradiated conditions. The analysis is monitored with a Cosmosil 5C_{18}-AR column using a mobile phase of CH_3CN–H_2O–AcOH (50:49:1, v/v/v) at 260 nm. The developed method satisfies the system suitability criteria, peak integrity, and resolution among the parent drug and its degradation products. The results indicate that the established assay method shows good selectivity and specificity suitable for stability measurements of CPF. CPF is found to be more sensitive to exposure to light and in acidic conditions, but it is stable in a basic medium. The kinetic study of the photodegradation of CPF follows an apparent first-order reaction in a variety of solvents. The solvent effects on the rates of degradation are in the decreasing order of chloroform > dichloromethane > methanol > ethanol > 2-propanol, which is irrelevant to the dielectric constant ε. However, the hydrogen-donating ability of the solvents is essential to the photochemical decomposition of CPF. A plot of log k versus the Kirkwood function exhibits a linear relationship in aqueous ethanolic solutions, which implies that degradation proceeds via an ionic mechanism.

Introduction

Carprofen (CPF), 2-(6-chloro-2-carbazolyl)propanoic acid, is a relatively new nonsteroidal anti-inflammatory drug (NSAID) that is used to treat patients with rheumatoid arthritis, osteoarthritis, and acute gouty arthritis (1–3). However, incidences of gastric irritation caused by these NSAIDs are frequently reported (4). The CPF that is available on the market is a solid; however, efforts to search for a new dosage form with better absorption characteristics in a rectal formulation (including aqueous solutions and suppository forms) have been under intensive investigation (5,6).

CPF has been reported to be very active in producing photosensitized allergic and hemolytic adverse effects (7–13). Therefore, the photostability of CPF must be seriously considered with respect to its safety and efficacy in formulations. Because stability studies are an important part of preformulation in dosage-form development, establishing a stability-indicating assay method suitable for potential critical factors has become an urgent task (14–16).

Assay methods for CPF in the form of solids or biological fluids using high-performance liquid chromatographic (HPLC) techniques have been studied with promising results (1,5,6,17,18). The objective of this study is to focus on the development of a rapid, sensitive, and accurate stability-indicating HPLC assay method to monitor the degradation of CPF under acidic, basic, or photo-irradiated conditions. In addition, we report on a series of kinetic studies of the photochemical decomposition of CPF in various solvent systems.

Experimental

Materials

CPF, indomethacin, and butylparaben were purchased from Sigma Chemicals (St. Louis, MO). 2,5-Dimethylfuran was from Acros Organics (Geel, Belgium). Liquid chromatographic (LC)-grade methanol, ethanol, and 2-propanol were from Fisher Chemicals (Springfield, NJ), and LC-grade chloroform, dichloromethane, and glacial acetic acid were from E. Merck (Darmstadt, Germany). A Cosmosil 5C_{18}-AR reversed-phase HPLC column was the product of Naikalai Tesque (Kyoto, Japan). All other chemicals were of reagent grade.

HPLC assay condition

A Shimadzu LC-10AT HPLC pump system equipped with an SPD-10A UV–vis detector, C-R6A integrator, and Jasco 851-AS autosampler was used to analyze the degraded samples on a

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Cosmosil $5C_{18}$-AR column (4.6- × 250-mm i.d.) at 260 and 270 nm. A mixture of CH_3CN–H_2O–HOAc (50:49:1, v/v/v) was used as the mobile phase at a flow rate of 1 mL/min. The injection volume was 20 µL, and indomethacin (IND) was used as an internal standard.

Degradation of CPF in acidic, basic, or photo-irradiated conditions

A 500-µg/mL ethanol solution of CPF was prepared as a stock solution. The stock solution was further diluted with 0.2 N HCl, 0.2 N NaOH, or distilled water in order to make an acidic, basic, or neutral solution, respectively, with a concentration of 100 µg/mL (i.e., each solution contained 20% ethanol). Six milliliters of each solution was transferred to a 20-mL clear glass container. The acidic and basic solutions were incubated for 3 days at 60°C, whereas the neutral solution was exposed to daylight for 10 min. The samples were then subjected to HPLC analyses.

Validation of HPLC method

The system suitability parameters—including capacity factor (k'), selectivity (α), resolution (R_s), plate number (N), and asymmetric factor (A_s)—of the HPLC system were established to adequate levels (19,20). The peak specificity of CPF was evaluated by comparing the ratio of the amount determined at two different wavelengths (260 and 270 nm). The linearity of CPF was carried out in the range of 2.5 to 80 µg/mL in ethanol containing 40 µg/mL of IND as an internal standard. The calibration curve was constructed by plotting the CPF–IND response area ratio versus concentration. The Lack-of-Fit test was used to confirm the adequacy of the regression model (19,21). The precision of the method was assessed by intra- and interday variabilities at a usual working concentration (50 µg/mL) with six replicate determinations for three consecutive days. The accuracy of the method was evaluated by the recovery test. The mimic excipients (starch–talc, 95:5, w/w) were compounded, and then 20 mg of the excipients was transferred to three individual 50-mL volumetric flasks. The 5-, 30-, and 60-µg/mL CPF ethanolic solutions containing 40 µg/mL of IND were prepared by adding adequate stock solutions of CPF and IND and then made to mark with ethanol. After ultrasonication for 10 min and filtration through a Millipore membrane (0.45 µm), the filtrate was subjected to HPLC analysis.

Kinetic studies of CPF

CPF at 50 µg/mL was prepared in methanol; ethanol; 2-propanol; dichloromethane; chloroform; and 80%, 60%, 40%, and 20% aqueous ethanolic solutions for the purpose of studying the kinetic behavior with respect to solvent effects. Each test solution (6 mL) was transferred to a 20-mL clear glass container and exposed to a fluorescent light (NEC-FL20SSEX-D/18HG). The distance from the light source to the samples was 30 cm (1500 lx). An aliquot of 500 µL solution was removed at each predetermined checkpoint. The remaining CPF in the solution was assayed with the established stability-indicating HPLC assay method.

Data analysis

The apparent first-order degradation rate constant was calculated by linear regression analysis using Sigmaplot version 2.01 for Windows from Jandel Scientific (San Rafael, CA).

Results and Discussion

Degradation of CPF

The chromatograms of the CPF that was degraded in acidic, basic, or photo-irradiated conditions are shown in Figures 1B, 1C, and 1D, respectively. Apparently, CPF was degraded to numerous products, especially under daylight exposure. The retention time of CPF was found to be 8.91 min (Figure 1A),

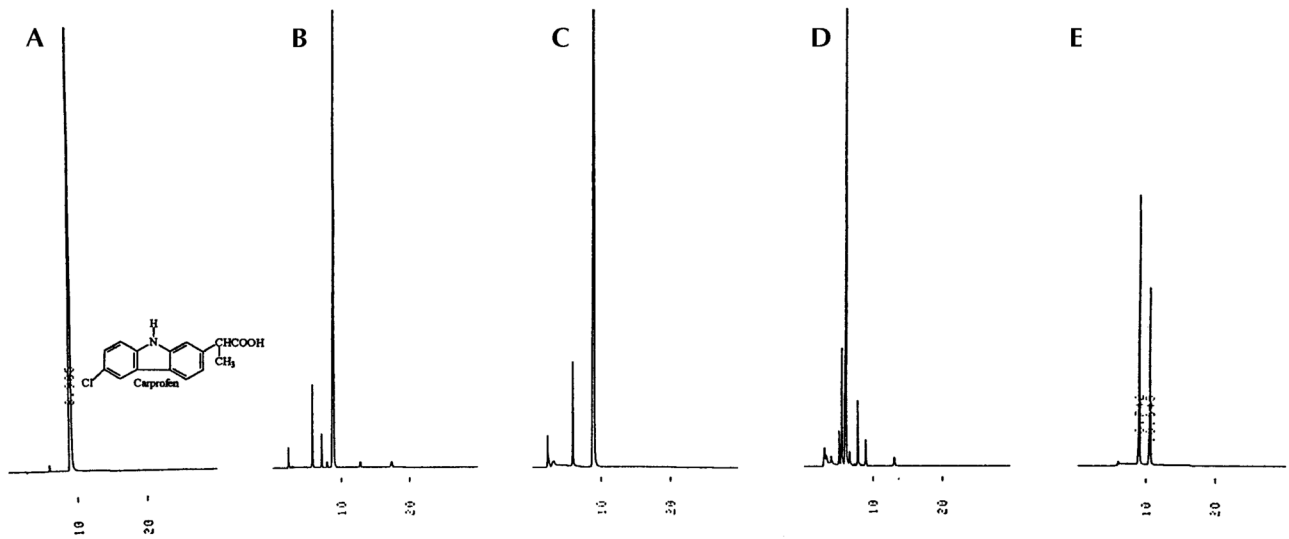

Figure 1. HPLC chromatograms of CPF: standard solution, A; degraded under acidic conditions for 3 days at 60°C, B; degraded under basic conditions for 3 days at 60°C, C; photodegraded in daylight for 10 min, D; and CPF and IND as internal standards, E.

and most of the degradation products had retention times shorter than that of CPF, indicating that the degradation products were more polar than the parent drug. To avoid interference by the degradation products, IND with a retention time of 10.35 min was chosen as an internal standard, which appeared after the parent drug (Figure 1E). Under acidic or basic conditions of incubation for 3 days at 60°C, the amounts of CPF remaining were 65.2% and 99.9%, respectively, whereas under daylight exposure for 10 min, it was 2.1%. The results clearly show that CPF is more labile to light and in acidic conditions, but it is stable in a basic medium.

Table I. System Suitability Parameters for CPF

Parameter	CPF	IND	Preferable levels
k'	3.57	4.23	2 to 8
α	1.18		1.02 to 2.0
R_s	2.59 (CPF–IND) 2.22 (CPF–DP*) 4.54 (IND–DP)		> 1.5
A_s	1.08	1.12	0.9 to 1.3
N	72,319	87,278	

* DP, degradation products of CPF.

Table II. Peak Area Ratios Quantitated at 260 and 270 nm*

Condition	CPF at 260 nm/270 nm	IND at 260 nm/270 nm
Standard solution	2.505 ± 0.009	1.073 ± 0.038
Acidic medium	2.531 ± 0.039	1.082 ± 0.049
Basic medium	2.505 ± 0.015	1.136 ± 0.036
Daylight exposure	2.552 ± 0.046	1.097 ± 0.036

* Data represent the mean ± standard deviation (n = 3).

Validation of the HPLC method

The system suitability parameters—including capacity factor (k'), selectivity (α), resolution (R_s), plate number (N), and asymmetric factor (A_s)—are shown in Table I. Obviously, all values of the system parameters are located within adequate levels of an optimized HPLC condition (19,20). Table II shows the ratio of the amount quantitated at 260 and 270 nm (before and after the stress treatment). The results of the statistical comparison using one-way analysis of variance (ANOVA) are shown in Table III. The lack of significant differences between the four groups for CPF and IND is indicative of peak homogeneity. A stability-indicating method must selectively separate the parent drug from its potential impurities and degradation products. Otherwise, a nonspecific assay method cannot obtain reliable measurements of drug stability (15). Our established method satisfied the system suitability criteria, peak integrity, and resolution among the parent drug, internal standard, and degradation products. The results clearly indicated that the established assay method has good selectivity and specificity for the stability measurements of CPF.

Table III. Comparison Between the Peak Area Ratios of CPF Determined at 260 and 270 nm

Component	Source of variation	d.f.*	SS†	MS‡	F_{ratio}
CPF	Between groups	3	0.0047	0.0016	1.5909§
	Within groups	8	0.0079	0.0010	
	Total	11	0.0126		
IND	Between groups	3	0.0069	0.0023	1.4225§
	Within groups	8	0.0013	0.0016	
	Total	11	0.0199		

* d.f., degrees of freedom.
† SS, sum of squares.
‡ MS, mean square.
§ $F_{ratio} < F_{(3,8,0.95)}$; differences between groups are not significant.

The linearity of the calibration curve was checked over the range of 2.5 to 80 µg/mL in ethanol containing 40 µg/mL IND as an internal standard. The calibration curve was constructed by plotting the CPF–IND response area ratio versus the concentration. The calibration curve for CPF was rectilinear in the concentration range studied. The correlation coefficient of the linear regression analysis was greater than 0.9999. The results of linear regression gave the equation $y = 0.03124x - 0.00045$ with 95% confidence limits for the intercept from –0.00621 to 0.00531 and the slope from 0.03109 to 0.03139. The difference of the intercept from zero was found not to be significant ($p > 0.05$). The ANOVA for testing the significance of regression is shown in Table IV. The F ratios for regression and Lack-of-Fit test confirm both the significance and the adequacy of the linear model. The intra- and interday relative standard deviations (RSDs) of six replicate

Table IV. Analysis of Variance of the Calibration Curve

Source of variation	d.f.*	SS†	MS‡	F_{ratio}
Regression	1	25.7453	25.7453	172787.2483§
Residual	34	5.0656×10^{-3}	1.4900×10^{-4}	
Lack-of-Fit	4	9.2831×10^{-4}	2.3208×10^{-4}	1.6828**
Pure error	30	4.1373×10^{-3}	1.3791×10^{-4}	
Total	35	25.7504		

* d.f., degrees of freedom.
† SS, sum of squares.
‡ MS, mean square.
§ $F_{ratio} > F_{(1,34,0.95)}$; regression is significant.
** $F_{ratio} < F_{(4,30,0.95)}$; there is no reason to doubt the linearity.

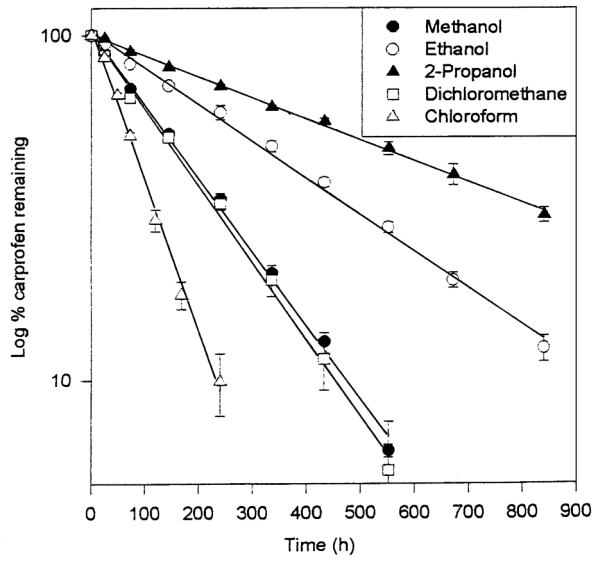

Figure 2. Apparent first-order rate plots of the photochemical decomposition of CPF in five organic solvents.

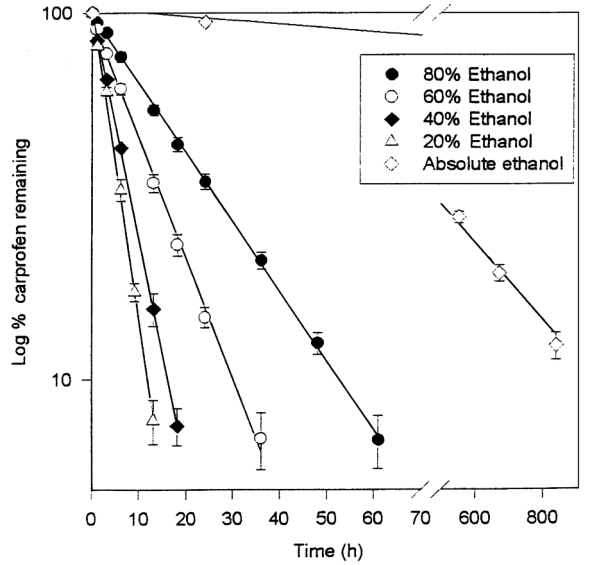

Figure 3. Apparent first-order rate plots of the photochemical decomposition of CPF in aqueous ethanolic solutions.

Table V. Observed Apparent First-Order Rates of Photochemical Decomposition of CPF in Nine Different Solvents

Solvent	k_{obs} (h^{-1}) × 10^3	$t_{1/2}$ (h)
2-Propanol	1.403	493.942
Ethanol	2.447	283.204
Methanol	4.912	141.083
Dichloromethane	5.125	135.220
Chloroform	10.096	68.641
80% Ethanol	43.483	15.937
60% Ethanol	75.500	9.179
40% Ethanol	133.608	5.187
20% Ethanol	197.253	3.513

Table VI. Relationship Between log k and the Kirkwood Function*

Solvent	ε	f_e	k_{obs} (h^{-1}) × 10^3
Absolute ethanol	24.5	0.470	2.447
80% ethanol	35.3	0.479	43.483
60% ethanol	46.1	0.484	75.500
40% ethanol	56.8	0.487	133.608
20% ethanol	67.6	0.489	197.253
Chloroform	4.8	0.358	10.096
Dichloromethane	8.9	0.420	5.125
Methanol	32.7	0.477	4.912
2-Propanol	19.9	0.460	1.403

* $f_e = (\varepsilon - 1) / (2\varepsilon + 1)$.

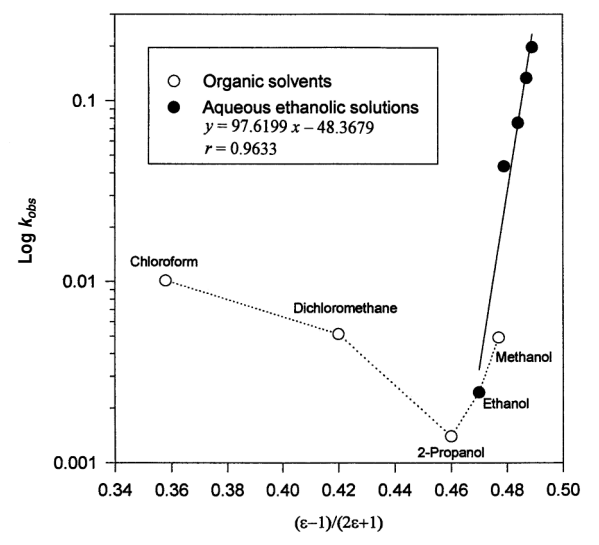

Figure 4. Correlation between log k and the Kirkwood function for the photochemical decomposition of CPF in aqueous ethanolic solutions.

determinations for three consecutive days at a usual working concentration of 50 µg/mL were 0.87% and 1.12%, respectively. The accuracies of the method—referring to the recovery test at the three concentrations of 5, 30, and 60 µg/mL (expressed as the closeness of the observed mean to the true value)—were determined to be 99.5%, 101.3%, and 98.9%, respectively. There was no significant difference in comparison with the results with 100% recovery ($p > 0.05$), which indicates good accuracy for the assay method. Clearly, the established assay method is reliable and applicable for the stability assessment of CPF degraded under acidic, basic, or photo-irradiated conditions.

Kinetic studies of photochemical decomposition of CPF in nine different solvents

The influence on the photochemical decomposition of CPF was investigated in nine different solvent systems—methanol; ethanol; 2-propanol; dichloromethane; chloroform; and 80%, 60%, 40%, and 20% aqueous ethanol. Plots of the logarithm of the percentage parent compound remaining versus time

(Figures 2 and 3) were linear ($r > 0.995$), indicating that the decomposition followed an apparent first-order reaction. The rate constants (k_{obs}) and the half-lives ($t_{1/2}$) in nine solvents were calculated and are shown in Table V. The photodegradation rates of CPF are in the decreasing order of chloroform > dichloromethane > methanol > ethanol > 2-propanol, which is irrelevant to the polarity or dielectric constant (ε) of the solvents, but is in good agreement with the order of their hydrogen-donating abilities. The influence of water contents on the decomposition rates in an aqueous ethanolic solution is shown in Figure 3. With an increase in water content, the photodegradation rate of CPF can be greatly accelerated. The formation of 2-(2-carbazolyl)propanoic acid via the abstraction of chlorine could not occur in the absence of hydrogen-donor molecules such as water (12). In an attempt to correlate log k versus the Kirkwood function (Table VI) using the Kirkwood–Onsager equation (22), a linear relationship was observed (Figure 4). Therefore, we expect that the degradation reaction in an aqueous medium proceeds by an ionic mechanism, which is quite different from that in hydrogen-donating organic solvents. The photochemical decomposition of CPF was previously reported to involve mainly a free radical pathway in order to produce 2-(2-carbazolyl)propanoic acid via the abstraction of chlorine in hydrogen-donating solvents accompanied by small amounts of oxidation products (12). CPF probably photodegrades via either the decarboxylation or abstraction of chlorine, which is in accordance with the apparent first-order kinetics found in the present study.

In conclusion, a solvent system with a stronger hydrogen-donating ability seems to dominate the photochemical decomposition rate of CPF. From a practical point of view, our findings suggest that using a solvent with a lower hydrogen-donating ability (such as 2-propanol) compounded with a decrease in water content when formulating pharmaceutical preparations of CPF would be an effective means of enhancing the photostability of the drug.

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