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Stability-indicating HPLC Assay Method of Zomepirac

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ABSTRACT

An HPLC assay method for determining the degradation of zomepirac was developed and validated under acidic, basic, and photo-irradiated conditions. The HPLC system consisted of an Inertsil 5 ODS-3V column (4.6 × 250 mm i.d.), and a guard column of Inertsil 7 ODS-3V (4.6 × 50 mm i.d.) using a mobile phase of CH_3CN: CH_3OH: 1%HOAc (2:64:34, v/v/v) with UV detection at 254 nm. The developed method satisfies the system suitability criteria, peak integrity, and resolution for the parent drug and its degradants. The results indicate that the established assay method shows good selectivity and specificity suitable for stability measurements of zomepirac. From the intra- and interday tests of 6 replicates, the coefficients of variations (CVs) were between 0.12% and 2.24% for the former and 0.15% and 3.93% for the latter. Recoveries were found to be between 97.14% and 101.58%. From the stress treatments, zomepirac was determined to be more sensitive to the light and acidic conditions, but it was stable in basic medium. A preliminary kinetic study of the photodegradation of zomepirac in methanol showed that it followed an apparent first-order reaction.

Key words: HPLC, zomepirac, photodegradation

INTRODUCTION

Zomepirac, 5-(*p*-chlorobenzoyl)-1,4-dimethylpyrrole-2-acetic acid sodium dihydrate, is a NSAID which gained reputation as a safe and effective non-narcotic analgesic[(1)]. Zomepirac was introduced in October 1980, but was withdrawn from the market in March 1983 because of serious side effects[(2)]. Numerous cases of zomepirac-induced anaphylactic shock have been reported[(3,4)]. There appear to be two mechanisms by which NSAIDs may cause anaphylactic symptoms. The first one is mediated by the NSAIDs inhibition of cyclooxygenase, the pivotal enzyme in prostaglandin synthesis[(5)]. The second one is an immunologically-mediated anaphylactic reaction after NSAID ingestion[(6)]. In addition, using *in vitro* assays based on red blood cell hemolysis and candida inhibition, majority of NSAIDs are shown to possess phototoxic properties[(7)]. Zomepirac, an acetic acid-derived NSAID containing a benzophenone and a diaryl ketone chromophores, mediates the development of phototoxic reactions[(8,9)]. Anaphylactic symptoms or phototoxicity due to zomepirac administration perhaps can be linked to the photodegradants produced when exposed to light.

A sensitive, specific, and rapid determination of zomepirac in human plasma and urine by a continuous body fluid monitoring system based on HPLC was reported by Müller and Zulliger[(10)]. However, the applicability of the existing HPLC methods on the samples containing photodegradants is still unclarified. It is therefore desirable to develop a stability-indicating HPLC assay method[(11-13)], which may enable simultaneous detection of acid-induced, base-induced and photo-induced degradants of zomepirac.

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MATERIALS AND METHODS

I. *Chemicals*

Zomepirac (Z) and butylparaben (BP) were purchased from Sigma Chemical (St. Louis, MO, USA). Methanol and acetonitrile of LC grade were from Merck (Darmstadt, Germany). Glacial acetic acid of reagent grade was the product of Ridel-deHaën (Seelze, Germany).

II. *HPLC Apparatus and Assay Conditions*

An Alcott 760 HPLC pump system (Norcross, GA, USA) equipped with a Jasco 875-UV detector (Tokyo, Japan), a CSW 1.7 integrator (Prague, Czech Republic), and an Inertsil 5 ODS-3V column (4.6 × 250 mm i.d., Vercopak Co., Taipei, Taiwan) equipped with a guard column of Inertsil 7 ODS-3V (4.6 × 50 mm i.d.) were used with a mobile phase of CH_3CN: CH_3OH: 1%HOAc (2:64:34, v/v/v). The UV detector was set at 254 nm and the flow rate was 1 mL/min. Butylparaben (BP) was used as an internal standard.

III. *Stress Treatment of Zomepirac in Acidic, Basic, or Photo-irradiated Conditions*

An amount of 17.45 mg (500 μM) of zomepirac and 9.75 mg of butylparaben (500 μM) as an internal reference were accurately weighed and placed in a 100-mL volumet-

ric flask. A concentration of 500 μg/mL methanol solution was prepared as a stock solution by adding the solvent to the marked volume. The stock solution was further diluted with 0.2 N HCl solution, 0.2 N NaOH solution, or distilled water, respectively, to make each solution with a concentration of 100 μg/mL in 20% methanol. Twenty milliliters of each solution was transferred to a 100-mL clear glass container. The acidic or basic solution was incubated at 60°C for 3 days, whereas the neutral solution was irradiated under a Hanovia 200-W high-pressure mercury lamp for 40 hr. The distance of the light source to the sample was maintained at 25 cm. The samples were then subjected to HPLC analysis. Each of the above 3 stress treatments was tested in triplicates.

IV. *Procedure in 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[11-13]. Peak specificity of zomepirac was evaluated by comparing the ratio of the amount determined at two different wavelengths of 254 and 270 nm. The linearity of zomepirac was carried out in the range of 5.0 to 100 μM in methanol containing 100 μM of butylpapraben as an internal standard. The calibration curve was constructed by plotting the zomepirac–butylparaben response area ratio vs. concentration. The Lack-of-Fit test was used to confirm the adequacy of the regression model[11-13]. The precision of the method was assessed by intraday and interday variabilities at the usual working concentrations of 5.0~100 μM with six replicate determinations for six consecutive days. The accuracy of the method was evaluated by recovery tests. The mimic excipients (starch/talc = 95/5, w/w) were compounded, and 20 mg of the excipients was then transferred to three individual 50-ml volumetric flasks. The 5.0~100 μM of a methanolic solution of zomepirac containing 100 μM of butylparaben were prepared by adding adequate stock solutions of zomepirac and butylparaben and made adequate amount of methanol to volume. After ultrasonication for 10 min and filtration through a Millipore membrane (0.45 μm) the filtrate was subjected to HPLC analysis.

V. *Photodegradation of Zomepirac*

For the purpose of analyzing photodegradants, 34.9 mg of zomepirac was weighed and placed in a 100-mL volumetric flask. Methanol was added to make the concentration of the sample to be exactly 1.0 mM. The sample was irradiated under a Hanovia 200-W high-pressure mercury lamp for 3 days. The distance from the light source to the samples was 25 cm (7200 lux). An aliquot of 20 μL solution was assayed with the HPLC method.

For stability experiment, 17.45 mg of zomepirac was accurately weighed and placed in a 100-mL volumetric flask. Methanol was added to volume and a 500 μM of the stock solution was prepared. Twenty milliliters of the solution was under irradiation by an Hg lamp as described above. The concentration of zomepirac remained was determined hourly by the newly developed HPLC method. The kinetic study was endured for 7 hr with triplicate measurements.

RESULTS AND DISCUSSION

I. *Degradation of Zomepirac*

The chromatograms of zomepirac degraded in acidic, basic, or photo-irradiated conditions (all in 20% aqueous methanol solution) are shown in Figure 1C, D, and E. The drug zomepirac was degraded to only one Z-A product in the acidic solution. Under the exposure of a high pressure Hg lamp, a total of 6 degradants were observed. Four degradants determined in pure methanolic solution are shown in Figure 1F with their retention times listed in Table 1. The retention time of zomepirac was found to be 17.78 min (Figure 1A). To avoid interference by the degradants, butylparaben with a retention time of 15.92 min was chosen as an internal standard (Figure 1B). After stress treatment under acidic or basic condition of incubation at 60°C for 3 days, the amounts of zomepirac remained were 50.2% and 96.9%, respectively whereas under Hg lamp irradiation for 40 hr, it was 25.5% (Table 2). The results clearly show that zomepirac is more labile to photo-irradiation than to acidic treatment. However, zomepirac is clearly stable in basic medium.

Table 1. HPLC retention time observed for zomepirac and the photodegradants Z-1, Z-2, Z-3 and Z-4

Degradant*	Retention time (min)
Z-1	4.72
Z-2	5.26
Z-3	10.50
Zomepirac	17.78
Z-4	39.03

*: Photo-irradiated for 3 days (Chromatogram shown in Figure 1F)

Table 2. Zomepirac remaining (%) after stress treatment

Condition	Ratio of zomepirac remaining (%)
Standard solution (control)	99.7 ± 0.1
Acidic solution	50.2 ± 2.1
Basic solution	96.9 ± 1.2
Light exposure (40 hr)	25.5 ± 4.5

Table 3. Peak area ratios of the amount of zomepirac determined before and after the stress treatment at 254 and 270 nm*

Condition	Zomepirac	Butylparaben
Standard solution	1.214 ± 0.0066	1.338 ± 0.0093
Acidic medium	1.237 ± 0.0156	1.340 ± 0.0054
Basic medium	1.242 ± 0.0253	1.368 ± 0.1023
Light exposure	1.238 ± 0.0095	1.348 ± 0.0247

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

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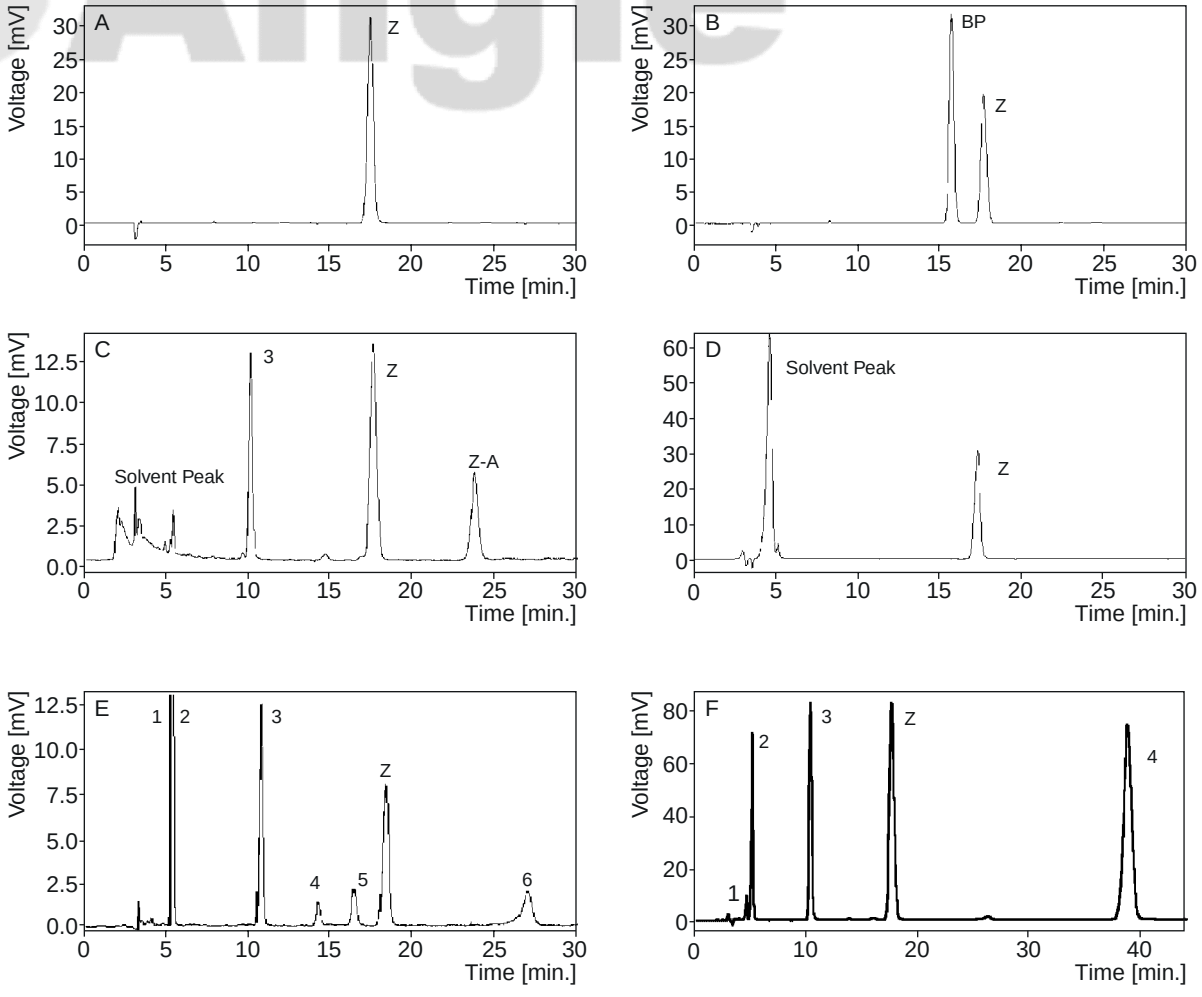

Figure 1. HPLC chromatograms of (A) standard solution of zomepirac; (B) zomepirac and butylparaben as internal standard; (C) degraded under acidic conditions for 3 days at 60°C; (D) degraded under basic conditions for 3 days at 60°C; (E) photodegraded by a high-pressure Hg lamp for 40 hr (in 20% aqueous CH_3OH); (F) photo-irradiated by a high-pressure Hg lamp for 3 days (in CH_3OH).

Table 4. System suitability parameters for zomepirac

Parameter	Zomepirac	Butylparaben	Preferable levels
k'	4.84	4.00	
α	1.21		> 1.02
R_s	3.7 (Z-BP§)		> 1.5
	3.46 (Z-PD*)		
	8.25 (Z-FD#)		
A_s	1.00	1.05	0.9 to 1.3
N	13043	11787	

§: Z-BP denotes zomepirac-butylparaben.
*: Z-PD denotes zomepirac-Z-3.
#: Z-FD denotes zomepirac-Z-4.

II. *Validation of HPLC Method*

The ratios of the amount of zomepirac determined before and after the stress treatment are shown in Table 3. 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 4. Obviously, all values of the system parameters are located within adequate levels of an optimized HPLC condition[11-13]. The results of statistical comparison using one-way ANOVA are shown in Table 5. The lack of significant differences

Table 5. Comparison between the peak area ratios of zomepirac determined at 254 and 270 nm by ANOVA analysis

Component	Source of variation	d.f.*	SS+	MS‡	F_{ratio}§
Zomepirac	Between groups	3	0.001441	0.00048	1.890205
	Within groups	8	0.002033	0.000254	
	Total	11	0.003474		
Butylparaben	Between groups	3	0.001658	0.000553	0.197343
	Within groups	8	0.022399	0.0028	
	Total	11	0.024056		

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

between the four groups for zomepirac and butylparaben is indicative of peak homogeneity. A quantitation method must selectively separate the parent drug from its potential impurities and degradants. Our established method satisfies the system suitability criteria, peak integrity, and resolution among the parent drug, internal standard, and degradants. The results clearly indicate that the established assay method has good selectivity and specificity for quantitation and stability measurements of zomepirac.

The linearity of the calibration curve was checked over the range of 5.0 to 100 μM in methanol containing 100 μM of butylparaben as an internal standard. The calibration curve was constructed by plotting the zomepirac–butylparaben response area ratio vs. concentration. The calibration curve for zomepirac is rectilinear in the concentration range studied. The related coefficient of the linear regression analysis is $r^2 > 0.999$. The results of linear regression give the equation $y = 0.028\ x + 0.0289$. The difference of the intercept from zero was found insignificant ($p > 0.05$). The analysis of variance for testing the significance of regression is shown in Table 6. The F ratios for regression and Lack-of-Fit test confirm both the significance and the adequacy of the linear model. The intraday (Table 7) and interday (Table 8) standard deviations (S.D.) of six replicate determinations for six consecutive days at the usual working concentrations of 5.0-100 μM were among 0.111 and 0.272 with CV between 0.12% and 2.24% for the former; 0.152 to 0.387 with CV between 0.15% and 3.93% for the latter. The accuracy of the method as referred by recovery tests at five concentrations (5, 10, 25, 50, and 100 μM), was determined to be 97.14%, 97.77%, 101.58%, 100.20% and 99.88%, respectively, indicating good accuracy for the assay method. Clearly, the established assay method is reliable and applicable for stability assessment of zomepirac degraded under photo-irradiated condition.

Table 6. Analysis of variance of the zomepirac calibration curve

Source of variation	d.f.*	SS+	MS‡	F_{ratio}
Regression	1	28.27418	28.27418	97793.7§
Residual	28	0.008095	0.000289	
Lack-of-Fit	3	0.000532	0.000177	0.585939£
Pure error	25	0.007564	0.000302	
Total	29	28.28228		

*d.f.: degrees of freedom

+SS: sum of squares

‡MS: mean square

§$F_{ratio} > F$: regression is significant

£$F_{ratio} < F$: there is no reason to doubt the linearity

Table 7. Intraday analytical precision and accuracy for zomepirac (n = 6)

Concentration (μM)	5	10	25	50	100
Y1	5.012	9.808	25.177	50.054	99.945
Y2	4.819	9.835	25.318	50.165	99.861
Y3	4.912	10.199	25.139	49.602	100.097
Y4	4.974	9.907	25.246	49.846	100.145
Y5	4.942	9.831	25.520	49.641	100.064
Y6	5.153	10.030	25.124	49.474	100.217
Mean	4.969	9.935	25.254	49.797	100.043
SD	0.111	0.152	0.148	0.272	0.129
CV (%)	2.24	1.53	0.58	0.54	0.12
Rel. err. (%)	-0.62	-0.65	1.01	-0.41	0.04

Table 8. Interday analytical precision and accuracy for zomepirac (n = 6)

Concentration (μM)	5	10	25	50	100
Y1	5.012	9.808	25.177	50.054	99.945
Y1	4.764	9.754	25.474	50.185	99.820
Y2	4.681	9.808	25.727	49.872	99.909
Y3	4.862	10.068	25.486	49.422	100.161
Y4	4.773	9.848	25.179	50.441	99.757
Y5	4.834	9.551	25.532	50.335	99.745
Y6	5.226	9.589	24.981	50.344	99.858
Mean	4.857	9.770	25.396	50.100	99.875
SD	0.191	0.187	0.268	0.387	0.152
CV (%)	3.93	1.92	1.05	0.77	0.15
Rel. err. (%)	-2.86	-2.23	1.58	0.2	-0.12

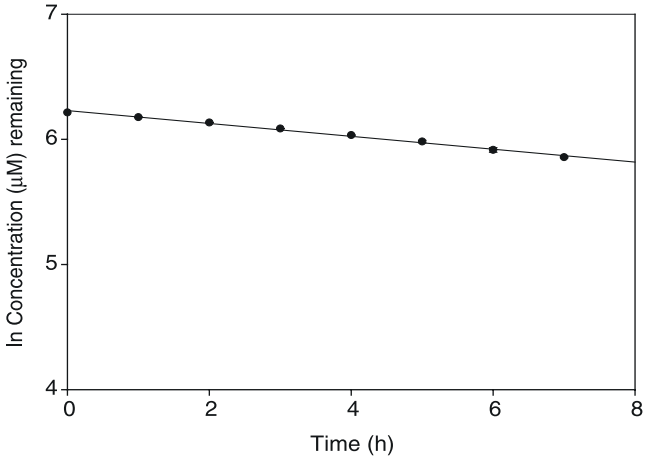

Figure 2. Apparent first-order rate plots of the photochemical decomposition of zomepirac in methanol.

III. *Preliminary Kinetic Study of Photodegradation of Zomepirac*

By applying the above developed and validated HPLC method for quantitation, the photodegradation of zomepirac starting from 500 μM in methanol was investigated and a total of 4 degradants were observed. A plot of the logarithm of the remaining parent drug (concentration in mM) vs. time (Figure 2) was linear. The linear equation of $y = -0.051\ x + 6.230$ ($r^2 > 0.993$) was obtained, indicating that the decomposition followed an apparent first-order reaction. The first-order rate constant is 5.1×10^{-2} (h^{-1}). The half-life, $t_{1/2}$, of consuming zomepirac is 815 min.

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