Quantitation of Tolmetin by High-Performance Liquid Chromatography and Method Validation

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Abstract

A high-performance liquid chromatographic (HPLC) assay method for assessing the degradation of tolmetin (TLM) is developed and validated under acidic, basic, and photoirradiated conditions. The HPLC method includes an Inertsil 5 ODS-3V column (250- × 4.6-mm i.d.), guard column of Inertsil 7 ODS-3V (50- × 4.6-mm i.d.), mobile phase of CH_3OH–1% HOAc (64:36, v/v), and 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 established assay method exhibits good selectivity and specificity suitable for stability measurements. From the intra- and interday tests of six replicates, the coefficients of variation are between 0.20% and 1.77% for the former, and 0.12% and 3.40% for the latter. Recoveries are found to be 98.7–101.7%. TLM is determined to be more reactive when exposed to light and acidic conditions, yet TLM is stable in a basic medium. A kinetic study of the photodegradation of TLM shows that it follows an apparent first-order reaction in three alcoholic solvents.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesics and antirheumatic agents (1). Tolmetin (TLM), 1-methyl-5-(*p*-toluoyl)-pyrrole-2-acetic acid sodium dihydrate was introduced into clinical practice in the United States in 1976. It is a relatively new NSAID that is widely used to treat juvenile rheumatoid arthritis. However, approximately 25–40% of patients taking TLM experience side effects, which are most commonly gastrointestinal (2). This drug has been reported to be involved in hemolytic (3) and anaphylactoid (4) reactions, which might be associated with light exposure. TLM is an acetic-acid-derived NSAID that also contains the benzophenone and diaryl ketone chromophores and mediates the development of phototoxic reactions. Although there is no clinical evidence for photosensitivity induced by TLM (5), this anti-inflammatory drug has been shown to produce phototoxicity when tested in vitro (2). There is speculation that the allergic dermatitis might be related to photodegradants of TLM. In fact, TLM had been studied in vitro in phosphate buffered solution irradiated with UVA light. It was found that the phototoxic properties of the drug are negligible in nitrogen and significant in aerated medium. The photolysis showed that TLM undergoes photodecarboxylation to *p*-toluoyl-1,2-dimethyl-5-pyrrolyl ketone in nitogen and *p*-toluoyl-1-methyl-2-hydroxymethyl-5-pyrrolyl ketone and 5-(*p*-toluoyl)-1-methyl-2-pyrrole carbaldehyde in air (4).

A rapid and sensitive high-performance liquid chromatography (HPLC) analytical procedure was reported for the simultaneous measurement of TLM in plasma and urine by Hyneck et al. (6). However, the applicability of existing HPLC methods for samples containing photodegradants is uncertain. It would therefore be desirable to develop an HPLC quantitative method that would enable the simultaneous detection of acid-, base-, and photodegradants of TLM.

Experimental

Materials

TLM and indomethacin (IND) were purchased from Sigma Chemical (St. Louis, MO). Liquid-chromatographic-grade methanol and acetonitrile were from Merck (Darmstadt, Germany). Reagent-grade absolute ethanol, isopropanol, and glacial acetic acid were products of Ridel-deHaën (Seelze, Germany).

HPLC apparatus and assay conditions

An Alcott 760 HPLC pump system (Norcross, GA) equipped with a Jasco 875-UV detector (Tokyo, Japan) set at 254 nm, CSW 1.7 integrator (Prague, Czech), and Inertsil 5 ODS-3V (250- × 4.6-mm i.d.) column (Vercopak, Taipei, Taiwan) equipped with a guard column of Inertsil 7 ODS-3V (50- × 4.6-mm i.d.) were used with a mobile phase of CH_3OH–1% HOAc (64:36, v/v). IND was used as an internal standard.

Irradiation conditions

A Hanovia 200-W high-pressure quartz mercury lamp (No. 654A-0360) with major emission lines at 313, 365, 436, and 546 nm was used as a light source. Irradiation was performed using a

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photochemical reactor (Model PR-2000, Panchum, Taipei, Taiwan) with the Hg lamp mounted overhead 30 cm from the sample. The light intensity of the radiation was measured using a ferric oxalate actinometer (7). The photon flux incident on the 5-mL solution in a quartz cuvette was of the order of 9×10^{14} quanta/s.

Stress treatment of TLM in acidic, basic, or photoirradiated conditions

Amounts of 15.75 mg (500µM) of TLM and 17.85 mg of IND (500µM) as an internal reference were accurately weighed and placed in a 100-mL volumetric flask. A 500-µg/mL stock solution was prepared by adding methanol to the mark. The stock solution was further diluted with 0.2N HCl, 0.2N NaOH, 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 1.5 h. The samples were then subjected to HPLC analyses.

Validation of the HPLC method

The system suitability parameters, including the capacity factor (k'), selectivity (a), resolution (R_s), plate number (N), and asymmetric factor (A_s), of the HPLC system were established at adequate levels (8–10). The UV spectrum of TLM shows three absorption maxima at 205, 254, and 316 nm. Specificity requires the analytical system to provide separation of the analyte from process impurities. Thus, peak specificity of TLM was evaluated by comparing the ratio of the amount determined at two different wavelengths (a maximum at 254 nm and a shoulder at 270 nm). The linearity of TLM was assessed over the range of 5.0–100µM in methanol containing 100µM of IND as an internal standard. The calibration curve was constructed by plotting the TLM–IND response area ratio versus concentration. Five standard solutions were used and the concentrations were 5.0, 10, 25, 50, and 100µM. Six replicate injections were made at random. The lack-of-fit test was used to confirm the adequacy of the regression model (8). The precision of the method was assessed by intra- and interday variabilities at the usual working concentrations of 5.0, 10, 25, 50, and 100µM with 6 replicate determinations for 6 consecutive days. The accuracy of the method was evaluated by the recovery test. Mimic excipients (starch/talc = 95/5, w/w) were compounded, and then 20 mg of the excipients was transferred to 3 individual 50-mL volumetric flasks. The 5.0–100µM TLM methanolic solutions containing 100µM of IND were prepared by adding adequate stock solutions of TLM and IND, which were then filled to the mark with methanol. After ultrasonication for 10 min and filtration through a Millipore membrane (0.45 µm), the filtrate was subjected to HPLC analysis.

Table I. Peak-Area Ratios of TLM Quantitated at 254 and 270 nm*

Condition	TLM at 254 and 279 nm	IND at 254 and 270 nm
Standard solution	1.072 ± 0.0052	1.064 ± 0.0013
Acidic medium	1.068 ± 0.0072	1.067 ± 0.0265
Basic medium	1.074 ± 0.0065	1.075 ± 0.0058
UV light exposure	1.073 ± 0.0144	1.064 ± 0.0127

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

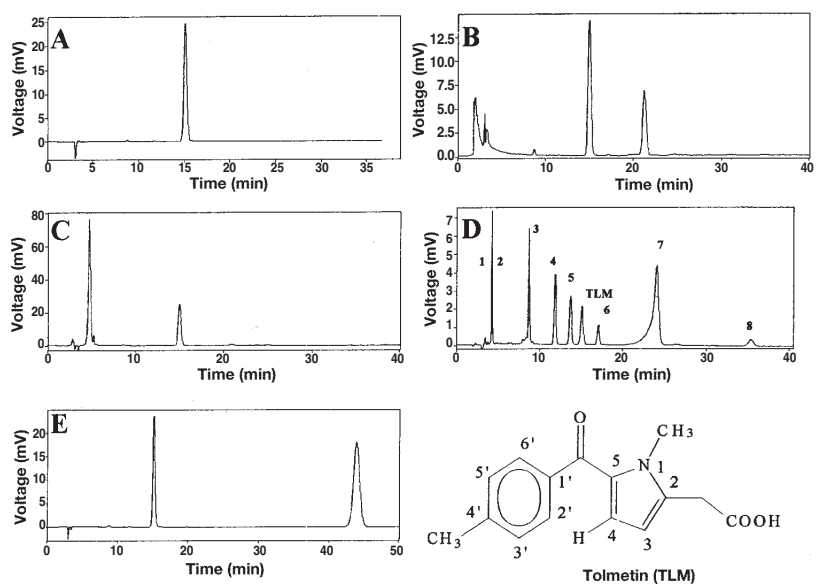

Figure 1. HPLC chromatograms of TLM: (A) standard solution, (B) degraded under acidic conditions for 3 days at 60°C, (C) degraded under basic conditions for 3 days at 60°C, (D) photodegraded by a high-pressure Hg lamp for 1.5 h (the eight degradants are numbered and arranged in increasing order of retention times), and (E) TLM with IND as the internal standard.

Photodegradation of TLM

An amount of 0.315 g of TLM was weighed and placed in a 100-mL volumetric flask. Methanol, ethanol, or isopropanol was added separately to make the concentration of the sample exactly 10mM. The sample was irradiated with a Hanovia 200-W high-pressure mercury lamp for 6 h. An aliquot of 20 µL was removed from the solution at each predetermined checkpoint at an interval of 1 h. The remaining TLM in the solution was assayed with the HPLC assay method.

Results and Discussion

Degradation of TLM

The chromatograms of TLM degraded in acidic, basic, or photoirradiated conditions are shown in Figure 1A–D. TLM was degraded to numerous products, especially under high-pressure Hg lamp exposure; 8 degradants were observe with their retention times in increasing order of **1**, 6.35; **2**, 6.36; **3**, 11.71; **4**, 14.91; **5**, 16.78; **6**, 18.77; **7**, 25.53; and 8, 34.20 min. The retention time of TLM was found to be 17.91 min (Figure 1A). To avoid interference by the degradants, IND with a retention time of 43.57 min was chosen as an internal standard (Figure 1E). With stress treatment under acidic or basic incubation condition at 60°C for 3 days, the amounts of remaining TLM were 62.4% and 98.1%, respectively; whereas under Hg lamp irradiation for 1.5 h, the amount was 7.9%. The results clearly show that TLM is more labile to

photoirradiation than to acidic conditions, but TLM is clearly stable in basic medium.

Table II. System Suitability Parameters for TLM

Parameter	TLM	IND	Preferable levels
k'	3.86	13.15	
a	3.40		> 1.02
R_s	22.03 (TLM–IND)		> 1.50
	2.20 (TLM–DP*)		
	3.25 (TLM–DP†)		
As	1.043	0.993	0.9–1.3
N	107820	114113	

* The preceding degradant of TLM.
† The following degradant of TLM.

Table III. Comparison between the Peak-Area Ratios of TLM Determined at 254 and 270 nm by ANOVA

Component	Source of variation	DF	SS	MS	F_{ratio}
TLM	Between groups	3	0.000074	0.000025	0.300093
	Within group	8	0.000659	0.000082	
	Total	11	0.000734		
IND	Between groups	3	0.000254	0.000085	0.376642†
	Within group	8	0.001798	0.000225	
	Total	11	0.002052		

* Abbreviations: DF, degrees of freedom; SS, sum of squares; and MS, mean square.
† $F_{ratio} < F_{(3,8,0.95)}$; difference between groups is not significant.

Table IV. ANOVA of the TLM Calibration Curve

Source of variation	DF	SS	MS	F_{ratio}
Regression	1	3.001035	3.001035	153807.7*
Residual	28	0.000546	0.0000195	
Lack-of-fit	3	0.00003925	0.00001308	0.645011†
Pure error	25	0.00050708	0.00002028	
Total	29	3.001582		

* $F_{ratio} > F$; regression is significant.
† $F_{ratio} < F$; no reason to doubt the linearity.

Table V. Intra- and Interday (n = 6) Analytical Precisions for TLM

	Intraday			Interday		
Concentration (µM)	Mean (SD)	CV (%)	Rel. error (%)	Mean (SD)	CV (%)	Rel. error (%)
5	4.909 (0.087)	1.77	–1.82	4.937 (0.168)	3.40	–1.26
10	10.003 (0.157)	1.57	0.00	10.173 (0.194)	1.91	1.73
25	25.219 (0.255)	1.01	0.87	24.928 (0.293)	1.17	–0.29
50	49.843 (0.518)	1.03	–0.31	49.918 (0.273)	0.54	–0.16
100	100.023 (0.201)	0.20	0.02	100.041 (0.129)	0.12	0.04

Validation of the HPLC method

Table I shows the ratio of the amount quantitated at 254 and 270 nm (before and after of the stress treatments). The system suitability parameters, including the capacity factor (k'), selectivity (a), resolution (R_s), plate number (N), and asymmetric factor (A_s), are shown in Table II. Obviously, all values of the system parameters are within adequate ranges of optimized HPLC conditions (8–10). The results of a statistical comparison using one-way analysis of variance (ANOVA) are shown in Table III. The lack of significant differences between the four groups for TLM and IND is indicative of peak homogeneity. A quantitative 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 TLM.

The linearity of the calibration curve was checked over a range of 5.0–100µM in methanol containing 100µM of IND as an internal standard. The calibration curve was constructed by plotting the TLM–IND response area ratio versus concentration. The calibration curve for TLM was rectilinear in the concentration range studied. The related coefficient of the linear regression analysis was greater than $r^2 = 0.999$. The results of linear regression gave the equation $y = 0.0093 + 0.0091x$. The difference of the intercept from zero was found to be nonsignificant ($p > 0.05$). The ANOVA for testing the significance of the regression is shown in Table IV. The F ratios for regression and lack-of-fit test confirm both the significance and adequacy of the linear model. The intra- and interday (Table V) standard deviations (SDs) of six replicate determinations for six consecutive days at the usual working concentrations of 5.0–100µM were between 0.087 and 0.518 with coefficients of variation (CVs) of between 0.20% and 1.77% for the former, and 0.129 to 0.293 with CVs of between 0.12% and 3.40% for the latter. The accuracies of the method, as referring to the recovery test at the 5 concentrations of 5, 10, 25, 50, and 100µM (expressed as the closeness of the observed mean to the true value), were determined to be 98.7%, 101.7%, 99.7%, 99.8%, and 100.0%, respectively. There was no significant difference in comparison with the results having 100% recovery ($p > 0.05$), which indicates good accuracy for the assay method. Clearly, the established assay method is reliable and applicable for stability assessment of TLM degraded under photoirradiated conditions.

Kinetic studies of photodegradation of TLM

The influence of three different alcoholic solvents on the photodegradation of TLM was investigated, and also the influence in the order of methanol, ethanol, and 2-propanol. Plots of the logarithm of the percentage of parent drug remaining versus time (Figure 2) were all linear. The linear equations of $y = –0.473x + 4.748$, $y = –0.654x + 4.711$, and $y = –0.695x + 4.722$ ($r^2 > 0.987$, 0.990, and 0.990, respectively) were obtained, indicating that the decomposition followed an apparent first-order reaction. The first-order rate constants were 0.473, 0.654,

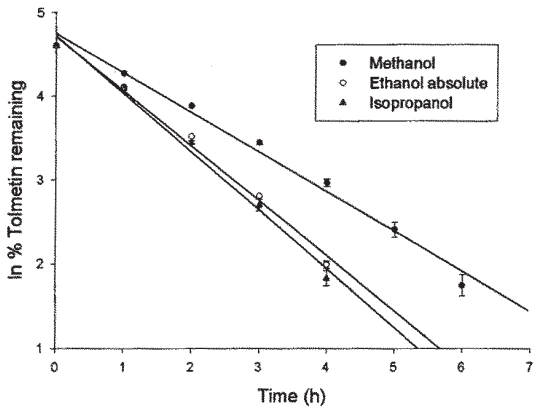

Figure 2. Apparent first-order rate plots of the photochemical decomposition of TLM in three alcoholic solvents.

and 0.695 h^{-1}, which are inversely proportional to the dielectric constants (ε = 32.7, 24.4, and 19.9) of the solvents. The results imply that the photodegradation of TLM proceeds via a free-radical process, which excludes the possibility of an ionic mechanism.

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References

1. J.E. Squires, P.D. Mintz, and S. Clark. Tolmetin-induced hemolysis. *Transfusion* **25(5):** 410–413 (1985).
2. L. Becker, B. Eberlein-König, and B. Przybilla. Phototoxicity of non-steroidal anti-inflammatory drugs: *in vitro* studies with visible light. *Acta Derm. Venereol.* **76(5):** 337–40 (1996).
3. A.C. Rossi and D.E. Knapp. Tometin-induced anaphylactoid reaction. *N. Engl. J. Med.* **307:** 499–500 (1982).
4. S. Giuffrida, G. De Guidi, S. Sortino, R. Chillemi, L.L. Costanzo, and G. Condorelli. Molecular mechanism of drug photosensitization. Part 6. Photohaemolysis sensitized by tolmetin. *J. Photochem. Photobiol., B* **29(2-3):** 125–33 (1995).
5. F. Bosca and M.A. Miranda. Photosensitizing drugs containing the benzophenone chromophore. *J. Photochem. Photobiol., B* **43(1):** 1–26 (1998).
6. M.L. Hyneck, P.C. Smith, E. Unseld, and L.Z. Benet. High-performance liquid chromatographic determination of tolmetin, tolmetin glucuronide and its isomeric conjugates in plasma and urine. *J. Chromatogr. A* **420(2):** 349–56 (1987).
7. J. Calver and J.N. Pitts. *Photochemistry*. John Wiley, New York, NY, pp. 783–86 (1966).
8. H.C. Hsu and C.S. Chen. Validation of analytical methods: a simple method for HPLC assay method. *J. Food Drug Anal.* **2(3):** 161–76 (1994).
9. C. Ho and G.L. Chen. Stability-indicating high-performance liquid chromatographic assay methods for drugs in pharmaceutical dosage forms: part I. *J. Food Drug Anal.* **4(4):** 271–92 (1996).
10. C. Ho and G.L. Chen. Stability-indicating high-performance liquid chromatographic assay methods for drugs in pharmaceutical dosage forms: part II. *J. Food Drug Anal.* **5(1):** 1–24 (1997).

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