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Central European Journal of Chemistry
DOI: 10.2478/s11532-007-0026-8
Research article
CEJC 5(3) 2007 835–845

Adsorptive stripping voltammetric determination of the anti-inflammatory drug tolmetin in bulk form, pharmaceutical formulation and human serum

Amr M. Beltagi[1*], Mona A. El-Attar[2], Enass M. Ghoneim[2]

[1] *Department of Chemistry & Physics, Faculty of Education, Kafr El- Sheikh University, 33516 Kafr El-Sheikh, Egypt*
[2] *Analytical Chemistry Research Unit, Department of Chemistry, Faculty of Science, Tanta University, 31527 Tanta, Egypt*

Received 17 January 2007; accepted 2 April 2007

Abstract: The electro-reduction of tolmetin at the hanging mercury drop electrode was studied in different supporting electrolytes using cyclic voltammetry and square-wave stripping voltammetry techniques. Voltammograms of tolmetin exhibited a single well-defined 2-electron irreversible cathodic peak in media of pH < 4, which may be attributed to reduction of the >C=O double bond of the analyte molecule. Adsorption of tolmetin onto the surface of the hanging mercury electrode was identified and each adsorbed tolmetin molecule was found to occupy an area of 0.23 nm^2. A square-wave adsorptive cathodic stripping voltammetric procedure was described for the direct determination of tolmetin in bulk form and pharmaceutical formulation (Rumatol® capsules) with a limit of quantitation of 2×10^{-9} M and a mean percentage recovery of $98.35 \pm 1.21\%$ to 99.57 ± 1.23. Moreover, the described procedure was successfully applied for the direct assay of tolmetin in spiked human serum without pretreatment or extraction prior to the analysis while a quantitation limit of 5×10^{-9} M tolmetin was achieved.

Keywords: Tolmetin, Rumatol capsule, spiked human serum quantification, square-wave adsorptive stripping voltammetry

1 Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are some of the most commonly prescribed medications for treatment of soft-tissue disorders associated with pain and in-

* E-mail: ambeltagi@hotmail.com

flammation [1]. Tolmetin, 1-Methyl-5-p-toluoylpyrrole-2-acetic acid, is a NSAID drug that exhibits considerable analgesic, anti-inflammatory and antipyretic efficacy [2] and it has proved to be effective for the treatment of rheumatoid arthritis in humans.

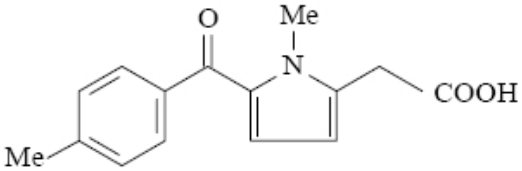

Fig. 1 Structure of tolmetin molecule.

Several methods for determination of tolmetin in pharmaceutical formulation and human fluids, including spectrophotometry [3, 4], supercritical fluid chromatography [5], micellar liquid chromatography [6] high performance liquid chromatography [7–12], electron-capture gas chromatography [13], liquid chromatography [14], and differential-pulse polarography [15], have been reported in the literature. The reported chromatographic methods for quantitation of tolmetin require sample pretreatment or time-consuming extraction prior to the analysis, as well as expensive reagents and equipment, which are too expensive for routine pharmaceutical analysis. Although adsorptive stripping voltammetry is a simple and extremely sensitive technique [16] it is not used to date for assay of tolmetin.

In this work, a square-wave adsorptive cathodic stripping voltammetric procedure was described for determination of tolmetin in bulk form, pharmaceutical formulations and spiked human serum.

2 Experimental

2.1 Solutions

A 1×10^{-3} M stock standard solution of bulk tolmetin (Sigma Pharmaceutical Industries, Quesna, Egypt) was prepared in methanol (Merck) and stored at 4 °C. Working solutions of tolmetin ($10^{-6} - 10^{-4}$ M) were prepared daily by appropriate dilution of the stock solution with methanol directly before use.

Rumatol® capsules (Sigma Pharmaceutical Industries, Egypt), with declared content of 200 or 400 mg tolmetin per capsule, were used in the present study. The content of five capsules (200 or 400 mg) was weighed and the average mass of capsule was determined. Then, they were grounded to a homogeneous fine powder. A quantity of the powder equivalent to 25 mg of tolmetin was accurately transferred to 70 ml of methanol (Merck) into a 100 ml volumetric flask, then sonicated for 15 min and the volume was made up to the mark with methanol. Afterwards, the solution was filtered through a 0.45 μm mili-pore filter (Gelman, Germany). Desired concentrations of tolmetin were obtained by accurate dilutions of the obtained solution with methanol. The solution was directly analyzed with the described square-wave adsorptive cathodic stripping voltammetric procedure.

Serum sample from a healthy volunteer was stored frozen until assay. To each of seven centrifugation tubes containing tolmetin at a certain concentration, a 1.0 ml of human serum and a 1.0 ml of methanol were added, and the contents were mixed well. Methanol was added to denature and precipitate the protein. After vortexing for 30 s, the precipitated protein was separated by centrifugation (Eppendorf Centrifuge, Model 5417 C, Hamburg, Germany) for 3 min at 14000 rpm. Clear supernatant was filtered through a 0.45 μm mili-pore filter to obtain protein-free human serum spiked with various concentrations of tolmetin. Spiked human serum sample was transferred carefully into a 10 ml volumetric flask and the volume was made up to the mark with the selected supporting electrolyte. The solutions were directly analyzed with the described square-wave adsorptive cathodic stripping voltammetric procedure without pretreatment and /or extraction prior to the analysis.

A series of the Britton-Robinson (B-R) universal buffer of pH $2-11$, hydrochloric and perchloric acid solutions of pH $1-2$ were prepared in deionized water and were used as supporting electrolytes. A pH - meter (Crison, Barcelona, Spain) was used for the pH measurements. Deionized water was supplied from a Purite-Still Plus Deionizer connected to Hamilton-Aqua Matic bidistillation water system (Hamilton Laboratory Glass LTD, Kent, UK).

2.2 Apparatus

Computer-controlled Electrochemical Analyzers Models 273A and 394-PAR (Princeton Applied Research, Oak Ridge, USA) controlled via 270/250 PAR software were used for voltammetric measurements. The electrode assembly (Model 303A- PAR) incorporating of a micro-electrolysis cell of a three electrode system comprising of a hanging mercury drop electrode (HMDE) as a working electrode (area: 0.026 cm^2), an Ag/AgCl (3 M KCl) reference electrode and a platinum wire auxiliary electrode, was used.

3 Results and Discussion

3.1 Cyclic voltammetric studies

Cyclic voltammograms of tolmetin in the B-R universal buffer (pH $2-11$), hydrochloric or perchloric acid solutions (pH 1-2) at the HMDE, exhibited a single 2-electron irreversible cathodic peak in media of pH < 4, which may be attributed to reduction of its >C=O double bond. The peak current intensity was much enhanced in perchloric acid solution of pH 2. The interfacial adsorptive character of tolmetin onto the HMDE was studied in perchloric acid solution of pH 2 by means of cyclic voltammetry. Figure 1 showed voltammograms of 1×10^{-6} M tolmetin in perchloric acid solution of pH 2 following its accumulation onto the HMDE under open circuit conditions (dotted curve) and then at -0.4 V for 30 s (scans 1 & 2). Significant enhancements of the peak current in the first scan (Figure 2, curve 1), compared to that in the subsequent scan at the same mercury drop

(curve 2) or to that recorded after accumulation under open circuit conditions (dotted curve), have confirmed adsorption of tolmetin onto the mercury electrode.

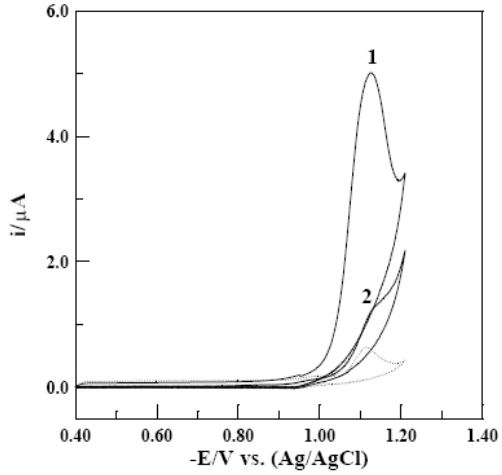

Fig. 2 Cyclic voltammograms for 1×10^{-6} M bulk tolmetin in a perchloric acid solution of pH 2 at $v = 0.2$ V s^{-1} after adsorptive accumulation onto the HMDE for 30 s: under open circuit conditions (dashed scan), at $E_{acc} = -0.4$ V (scans 1 & 2).

Adsorption of tolmetin was also identified by measuring its cyclic voltammetric peak current (i_p) in perchloric acid of pH 2 at increased scan rate v ($50 - 300$ mV s^{-1} after adsorptive accumulation onto the HMDE at - 0.4 V for 30 s. The $\log i_p$ versus $\log v$ plot was linear and the corresponding regression equation is $\log i_p = 0.90 \log v$ ($r = 0.997$ and $n = 6$). The slope of 0.90 is close to the expected theoretical value of 1.0 for ideal case of surface-adsorbed species [17] with some contribution from diffusion.

Surface coverage of the electrode, Γ^o (defined as the amount of reactant adsorbed onto the electrode surface, mol cm^{-2}) was calculated using the expression: $\Gamma^o = Q/nFA$, where (Q) is the amount of charge (μC) consumed in the surface process estimated from the integration of the voltammetric peak area [18], n is the total number of electrons consumed during reduction ($n = 2$), A is the surface area of the mercury electrode (0.026 cm^2). The electrode surface coverage was estimated as 6.9758×10^{-10} mole cm^{-2}. This means that each adsorbed tolmetin molecule occupies 0.23 nm^2.

3.2 Square-wave stripping voltammetric studies

3.2.1 Optimization of an analytical procedure

Square-wave voltammograms of 2×10^{-7} M bulk tolmetin in the B-R universal buffer of pH $2 - 4$, hydrochloric acid (pH $1 - 2$) or perchloric acid (pH $1 - 2$) solutions after adsorptive accumulation onto the HMDE at - 0.5 V for 30 s exhibited a single irreversible cathodic peak (Figure 3). A sharper and a much enhanced peak current were obtained in perchloric acid solution of pH 2 (Figure 3), therefore it was chosen as a supporting electrolyte throughout the further measurements in the present work.

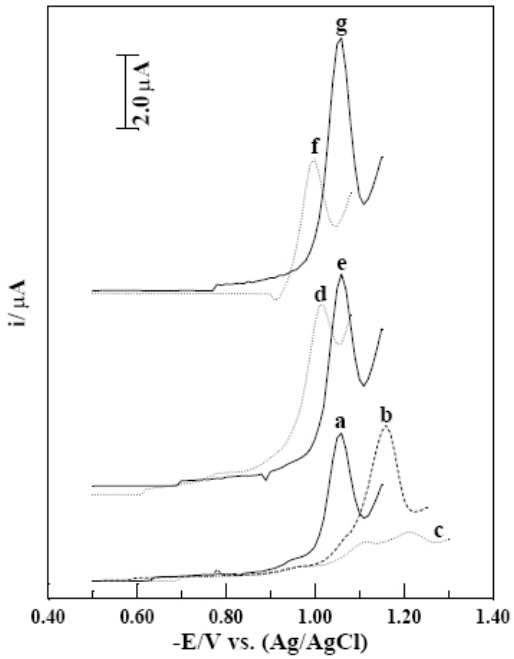

Fig. 3 SW-AdCS voltammograms for 2×10^{-7} M bulk tolmetin in a B-R buffer of pH: 2 (a), 3 (b) & 4 (c), a hydrochloric acid solution of pH : 1 (d) & 2 (e) and a perchloric acid solution of pH : 1 (f) & 2 (g); $E_{acc} = -0.5$ V, $t_{acc} = 30$ s; $f = 120$ Hz, $\Delta s = 10$ mV and $E_{sw} = 25$ mV.

The optimum instrumental conditions (frequency f, scan increment Δs and pulse-amplitude E_{sw}) were identified from the study of the peak current for 2×10^{-7} M tolmetin in perchloric acid solution (pH 2) after adsorptive accumulation onto the HMDE at -0.5 V for 30 s. At a scan increment of 10 mV and a pulse-amplitude of 25 mV, the peak current increased linearly over the frequency range $10 - 120$ Hz. At a frequency of 120 Hz and a pulse-amplitude of 25 mV, the peak current intensity increased linearly with the scan increment up to 12 mV. The optimal pulse-amplitude was also examined at $f = 120$ Hz and $\Delta s = 12$ mV and was found to increase linearly with pulse-amplitude up to 50 mV.

The effect of varying the accumulation potential (E_{acc}.) from 0.0 to -0.9 V on the peak current intensity of the square-wave cathodic adsorptive stripping (SW-AdCS) voltammogram for 2×10^{-7} M tolmetin in perchloric acid solution of pH 2 after adsorptive accumulation onto the HMDE for 30 s was also evaluated. At a potential of -0.4 V a much enhanced peak current was obtained (Figure 4). At lower and higher potentials the peak current decreased. This decrease might be attributed to desorption of tolmetin at either higher or lower potentials with respect to the zero charge potential, which in turn corresponds to the strongest adsorption of uncharged organic molecules.

SW-AdCS voltammograms of 1×10^{-8} M, 5×10^{-8} M and 1×10^{-7} M tolmetin solutions were recorded under the optimal experimental operational conditions. After adsorption accumulation onto the HMDE at -0.4 V. As shown in Figure 5, the magnitude of the peak current depended linearly on both the analyte concentration and the accumulation time.

Apparently, lower concentration of the analyte requires longer adsorptive accumulation time. This meant that the choice of accumulation time was dictated by the sensitivity required. In the present electro-analytical procedure accumulation times of 60 and 150 s were used.

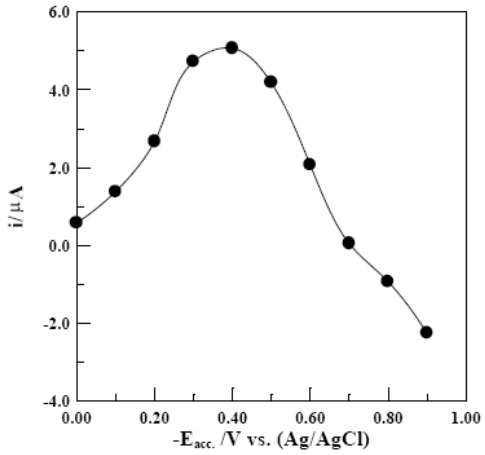

Fig. 4 Effect of accumulation potential (E_{acc}) on the SW-AdCS voltammetric peak current (i_p) for 2×10^{-7} M bulk tolmetin in percholric acid of pH 2 after adsorptive accumulation onto the HMDE for 30 s ($f = 120$ Hz, $\Delta s = 12$ mV and $E_{sw} = 50$ mV).

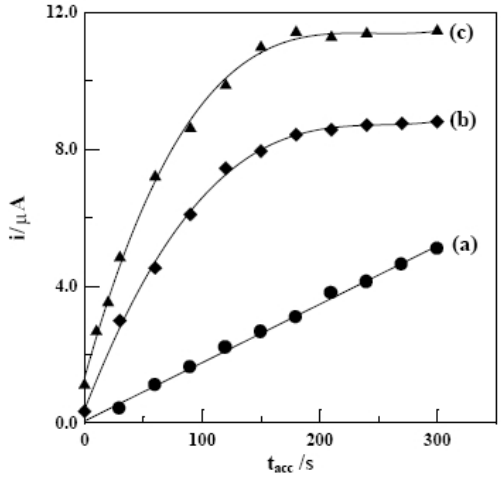

Fig. 5 Effect of accumulation duration (t_{acc}) on the SW-AdCS voltammetric peak current (i_p) for; (a) 1×10^{-8} M, (b) 5×10^{-8} M and (c) 1×10^{-7} M bulk tolmetin in percholric acid of pH 2 ($E_{acc} = -0.4$ V $f = 120$ Hz, $\Delta s = 12$ mV and $E_{sw} = 50$ mV).

On the other hand, SW signal increased with the increase of mercury electrode area (0.01 to 0.026 cm^2). In this study, HMDE of an area of 0.026 cm^2 was used. The influence of rest period [16] was also considered and a rest period of 5 s was applied. Accordingly, the optimal procedural conditions of the proposed procedure were: $E_{acc.} = -0.4$ V, $t_{acc} = 60 - 150$ s, $f = 120$ Hz, scan increment $\Delta s = 12$ mV, pulse-amplitude $E_{sw} = 50$

mV, area of the mercury electrode = 0.026 cm^2 , rest period = 5 s and a perchloric acid solution of pH 2 as a supporting electrolyte.

3.2.2 Method Validation

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. The elements required for method validation are: linearity range, limits of detection and quantitation, accuracy, precision, selectivity, robustness and interlaboratory reproducibility [19]. Under the optimal conditions of the proposed SW-AdCS voltammetric procedure, linear calibration plots were constructed over various concentration ranges and referred to different accumulation times (Table 1). Regression equations using the least square method corresponding to the calibration plots exhibited good linearity (Table 1), thus confirmed validity of the proposed procedure for determination of tolmetin. Limits of detection (LOD) and quantitation (LOQ) were calculated using the expression: $k\ SD/b$ [19], where $k = 3$ for LOD and 10 for LOQ, SD is the standard deviation of the intercept (or the blank) and b is the slope of the calibration curve. The achieved LOD of 6×10^{-10} M and LOQ of 2×10^{-9} M confirmed the sensitivity and validity of the proposed procedure for assay of tolmetin.

Table 1 Characteristics of calibration plots of SW-AdCS voltammetric determination of bulk tolmetin in a perchloric acid solution of pH 2.

$t_{acc.}$	Linearity range	Least square equation			LOD	LOQ
		Intercept	Slope			
(s)	(M)	(μA)	(μA/μM)	(r)	(M)	(M)
60	$1\times10^{-8}-4\times10^{-7}$	0.15	41.59	0.994	3×10^{-9}	1×10^{-8}
150	$2\times10^{-9}-5\times10^{-8}$	0.11	314.04	0.997	6×10^{-10}	2×10^{-9}

Repeatability of results using the proposed SW-AdCS voltammetric procedure was examined by performing five replicate measurements for 1×10^{-8} M tolmetin following accumulation onto the HMDE at −0.4 V for 60 s. A mean recovery of $98.35\pm1.21\%$ ($n = 5$) was achieved.

Voltammetric techniques have found widespread use in drug analysis, since the voltammetric procedures usually involve a simple dilution step and most of the excipients used do not interfere in the subsequent determination [20]. To identify the selectivity of the described SW-AdCS voltammetric procedure, analysis of 5×10^{-8} M of standard solution of bulk tolmetin (which contains no excipients) and 5×10^{-8} M standard solution of rumatol® capsules (which contains its excipients) were carried out after adsorptive accumulation of tolmetin onto the HMDE at −0.4 V for 60 s. The obtained recoveries and relative standard deviations in the absence ($98.35\pm1.2\%$) and presence (98.67 ± 1.68) of excipients indicated no significant interference from excipients. Thus, the described SW-AdCS voltammetric procedure can be considered selective.

Robustness [19] of analytical results indicates their resistivity to some changes of

experimental conditions. In the present work, the influence of small variation of pH (2 ± 0.5) and accumulation potential (-0.35 to -0.45 V) on recovery and standard deviation was examined by determination of 5×10^{-8} M tolmetin. As shown in Table 2, the achieved mean percentage recoveries were not significantly affected by variations of some of the experimental conditions. Consequently, the described SW-AdCS voltammetric procedure was regarded reliable for determination of tolmetin and can be considered robust.

Interlaboratory reproducibility of the proposed SW-AdCS voltammetric procedure was examined by determining 5×10^{-8} M tolmetin using two Potentiostats, Models 273A - PAR (Lab. 1) and 394 - PAR (Lab. 2), at different elapsed time. Mean percentage recoveries obtained in Lab.(1) and Lab. (2) , Table 2, as well as day- to-day recovery (98.5 ± 1.2 to 97.8 ± 1.4) were found reproducible, as there was no significant difference between recoveries of tolmetin or the standard deviation values.

Table 2 Influence of small variation in some of the experimental conditions of the proposed procedure on recovery of 5×10^{-8} M bulk tolmetin; (f = 120 Hz, Δs = 12 mV E_{sw} = 50 mV and $t_{acc.}$ = 60 s).

Variable	%R ± SD (n = 3)	Conditions
Robustness		
pH of the medium:*		
1.5	102.93 ± 2.84	$E_{acc} = -0.4$ V
2.0	98.35 ± 1.21	
2.5	97.42 ± 1.19	
*Accumulation potential (E_{acc})**		
-0.35 V	96.09 ± 1.64	pH= 2
-0.40 V	98.35 ± 1.21	
-0.45 V	96.11 ± 1.28	
Interlaboratory reproducibility	98.35 ± 1.21	pH= 2,
Potentiostat 273A-PAR	101.08 ± 0.89	$E_{acc} = -0.4$ V,
Potentiostat 394-PAR		

* Potentiostat 273 -PAR

3.3 Applications

3.3.1 Analysis of Rumatol® capsules

The optimized procedure was successfully applied to the direct determination of tolmetin in rumatol capsules (200 and 400 mg tolmetin per capsule) without pretreatment or extraction prior to the analysis. The results were obtained using the calibration plot. The obtained results (Table 3) were statistically compared with those obtained by a reported micellar LC method [6]. Since calculated value of F does not exceed the theoretical value (Table 3), there was no significant difference between the proposed and reported methods with respect to reproducibility [21]. Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by t-value [21], Table 3. The

Table 3 Assay of tolmetin in pharmaceutical formulations by the proposed SW-AdCS voltammetric procedure and a reported micellar LC method [6].

Sample	Claimed value (mg/Capsule)	Proposed procedure (% R ± SD) Calibration curve	Standard addition	Reported method [6] (% R ± SD)
Rumatol®	200	99.57 ± 1.23 $F = 4.30$ $t = 1.68$	100.88 ± 0.93	101.70 ± 2.55
	400	98.67 ± 1.68 $F = 3.19$ $t = 2.20$	98.33 ± 1.07	102.05 ± 3.00

Theoretical $F-value = 6.60$ and $t-test = 2.45$ at 95% confidence limit for $n_1 = 4$ and $n_2 = 4$

accuracy of the proposed procedure was also judged by applying the standard addition method [22].

3.3.2 Analysis of tolmetin in spiked human serum

Figure 6 illustrates the SW-AdCS voltammograms for various concentrations of tolmetin after adsorptive accumulation onto the HMDE at −0.4 V for 150 s. Variation of the peak current versus tolmetin concentration was linear within the concentration range $5 \times 10^{-9} - 7 \times 10^{-8}$ M, following the equation; i_p (μA) = 214.8 C (μM) + 0.692 ($r = 0.997$ and $n = 7$).

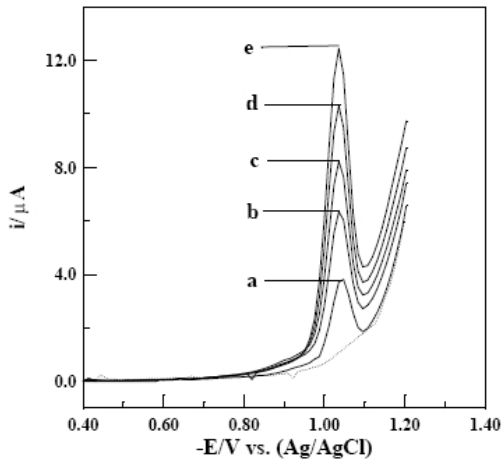

Fig. 6 SW-AdCS voltammograms for various concentrations of tolmetin spiked in human serum recorded after adsorptive accumulation onto the HMDE at $E_{acc} = -0.4$ V for $t_{acc} = 150$ s. The dotted line represents the background, (a) 1×10^{-8}, (b) 2×10^{-8}, (c) 3×10^{-8}, (d) 4×10^{-8} and (e) 5×10^{-8} M tolmetin ($f = 120$ Hz, $\Delta s = 12$ mV and $E_{sw} = 50$ mV).

A mean percentage recovery of tolmetin in human serum, based on the average of four replicate measurements, was found to equal 97.91 ± 1.88 without pretreatment or extraction prior to the analysis. The achieved LOD and LOQ of tolmetin spiked in human serum were 1.5×10^{-9} M (0.42 ng ml^{-1}) and 5×10^{-9} M (1.40 ng ml^{-1}), respectively.

4 Conclusions

A validated square-wave adsorptive cathodic stripping voltammetric procedure was described and successfully applied for assay of tolmetin in bulk form, pharmaceutical formulations and spiked human serum. The procedure was simple, sensitive, precise and showed clear advantages such as a short period of real time of analysis and no pretreatment or time-consuming extraction were required prior to the analysis. The procedure could be recommended for analysis of tolmetin in quality control and clinical laboratories.

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