Electro-oxidation of nimesulide at gold electrode and its determination in pharmaceutical dosage form and human biological fluid

"The truth is that all of us attain the greatest success and happiness possible in this life whenever we use our native capacities to their greatest extent."

~Smiley Blanton
7.1. INTRODUCTION

Nimesulide, N-(4-nitro-2-phenoxyphenyl)methanesulfonamide (Scheme 1), is a non-acidic (pKa = 6.5) non-steroidal anti-inflammatory drug (NSAID) with anti-pyretic, and analgesic properties. It inhibits prostaglandin synthetase/cyclooxygenase, which limits prostaglandin production. Its cyclooxygenase inhibiting potency is intermediate, but is relatively selective for the cyclo-oxygenase-2 (COX-2) thus the potential for gastric injury and intolerance is less. It is also a free radical scavenger, and helps to protect against the tissue damage that occurs during inflammation. It is effective in reducing the pain associated with osteoarthritis and rheumatoid arthritis.¹

![Scheme 1](image)

Techniques for detecting nimesulide include chromatography,²,³ electrochemical methods⁴,⁵ and spectrometry.⁶,⁷ However, chromatography and spectrometry require several time-consuming manipulation steps and expensive instruments. Electrochemical methods have been extensively employed as rapid, simple and accurate methods. Furlanetto et al.⁸ proposed adsorptive stripping voltammetry for the determination of nimesulide. But the utilization
of the mercury electrodes would contaminate the environment as a result of their environmental toxicity. Catarino et al.\textsuperscript{9} presented an amperometric method using a glassy carbon electrode for the determination of nimesulide, and the linear range was from $5.0 \times 10^{-5}$ to $3.0 \times 10^{-4}$ M. However, the method was only described for the determination of nimesulide in pharmaceutical formulations. Wang et al.\textsuperscript{4} used carbon nanotubes and cysteic acid based on electrochemical oxidation of L-cysteine to form a composite thin film material at a glassy carbon electrode.

The advance in experimental electrochemical techniques in the field of analysis of drugs is due to their simplicity, low cost and relatively short analysis time when compared with the other techniques. Electrochemical methods have proven to be useful for development of very sensitive and selective methods for the determination of organic molecules including drugs. In addition application of electro analytical techniques include the determination of electrode mechanisms. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmaceutical activity. Biotransformation pathways of nitro aromatic compounds are believed to result from nitro reductases that have the ability to use nitro as either one- or two- electron acceptors. The gold electrode has been widely used in electrochemical studies and electro analysis for various substrates for a long time because of its stability, wide potential window and fast electron transfer rate.\textsuperscript{10,11} To the best of our knowledge, till date there is no
report in literature on the electrochemical oxidation of nimesulide on gold electrode. The aim of this study is to establish the suitable experimental conditions, to investigate the electrochemical behavior, and oxidation mechanism of nimesulide on gold electrode by cyclic voltammetry. Further, differential pulse voltammetric (DPV) method with good precision and accuracy was developed for the determination of nimesulide in pharmaceutical formulations and in real samples in this chapter.

7.2. EXPERIMENTAL

7.2.1. Reagents and chemicals

Pure nimesulide in powdered form was obtained from Sigma-Aldrich and used as received. A stock solution of nimesulide (1.0 x 10^{-2} M) was prepared in HPLC grade methanol (S.D. Fine-Chem.). Rest of the reagents was same\textsuperscript{12} as given in Chapter VI (p. 199 and 200).

7.2.2. Instrumentation

Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., U.S.A.). The voltammetric measurements were carried out in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode as given in Chapter V (p. 171) and a 2 mm diameter gold electrode as a working electrode (Part No. CHI101).
The area of the electrode was obtained by cyclic voltammetric (CV) method from the slope of the plot of \( I_p \) versus \( \nu^{1/2} \) relation.\textsuperscript{13} In our experiment the slope was \( 7.59 \times 10^{-6} \, \mu A \, (Vs^{-1})^{-1/2} \) and the area of electrode was calculated to be 0.067 cm\(^2\).

### 7.2.3. Analytical procedure

The polishing of the working electrode was done on micro cloths (Buehler) glued to flat mirrors. A different micro cloth was used for each size of alumina. The particle size used was 0.3, 0.1 and 0.05 µm. The final particle size was 0.05 µm. After initial cleaning of the electrode, it was only necessary to polish with 0.05 µm particle size during the time of experiments. Before transferring the electrode to the solution, it was washed with double distilled water. Cyclic voltammograms were recorded in 0.2 M of pH = 6.5 at 50 mVs\(^{-1}\) between 0 and 1.4 V, until obtaining the reproducible current–potential curves. The experimental conditions for DPV were: initial potential: 0.4 V, final potential: 1.2 V, sensitivity: 5 µA/V, pulse amplitude: 50 mV, sample width: 20 ms, pulse width: 60 ms, pulse period: 200 ms and scan rate: 20 mVs\(^{-1}\).

### 7.2.4. Sample preparation

Two pieces of nimesulide containing tablets were weighed and ground to a homogeneous fine powder in a mortar. Portion equivalent to a stock solution of a concentration about 1.0 mM was accurately weighed and
transferred into a 100 ml calibrated flask and completed to the volume with methanol. The contents of the flask were sonicated for 10 min to get complete dissolution. Appropriate aliquot of the clear supernatant liquor was then transferred into a voltammetric cell containing 10 ml of buffer solution of pH 6.5. The differential pulse voltammogram was subsequently recorded following the optimized conditions. The content of the drug in tablet was determined referring to the calibration graph or regression analysis.

To study the accuracy of the proposed method, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of nimesulide to known concentration of the tablets. The resulting mixture was analyzed as in pure nimesulide.

7.3. RESULTS AND DISCUSSION

7.3.1. Cyclic voltammetric behavior of nimesulide

The electrochemical behavior of nimesulide at gold electrode was investigated using CV at pH = 6.5. The cyclic voltammograms obtained for 1.0 × 10⁻³ M nimesulide solution at a scan rate of 50 mVs⁻¹ exhibits a well-defined irreversible anodic peak. The results are shown in Figure VII (i) (p. 229). The voltammogram of blank solution was shown by curve (a) and anodic peak corresponding to nimesulide oxidation appeared at about 0.943 V as shown in
curve (b). The cathodic peak was appeared at 0.42 V corresponding to reduction of gold oxides.¹⁴

7.3.3. Influence of pH

The electrochemical responses of nimesulide in 0.2 M PBS with different pH values and at a scan rate of 0.05 Vs⁻¹ were studied. The peak current was highest at pH 6.5, as seen in Figure VII (ii a) (p. 230). So pH 6.5 PBS was used for the determination of nimesulide and for further experiments. With the increase in the solution pH, the peak potentials shifted to less positive values. This effect of pH on the electrochemical properties of soluble ions in solution can be attributed to the acid-base equilibrium constants of this drug. Gold electrodes are very weak chemisorbers due to filled d-orbitals, yet display a higher electroactivity towards drugs oxidation. The electrocatalytic behavior of gold is highly complex. The catalytic component of gold electrode is believed to be hydrous gold oxide, AuOH, which is formed by the chemisorption of hydroxide anions to the gold surface. This effect is more pronounced at higher pHs, and occurs in the region of premonolayer oxidation of the gold surface. This process occurs at potentials of -0.1 to 0.4 V vs. Ag/AgCl (3 M KCl) depending on the surface structure of the gold electrode. Therefore, the gold oxide formation and its reduction is pH dependent.¹⁵ Thus, an increase in pH induces a cathodic shift of the corresponding peaks.
The linear relation between $E_p$ and pH is as shown in Figure VII (ii b) (p. 230). A well-defined sharp oxidation peak was observed between pH 3.0 to 11.2 and above the pH 11.2, the oxidation peak was not so sharp. Hence, the pH study was restricted only from 3.0 to 11.2. The linear relationship between $E_p$ and pH can be expressed as follows:

$$E_p = 1.3007 - 0.0474 \text{ pH}; \ (E_p \text{ in V, } r = 0.9487)$$

From the plot of $I_p$ vs. pH (Figure VII (ii c) (p. 230)) it is clear that, peak current increased with increase in pH value.

7.3.4. Influence of scan rate

The electrochemical behaviors of nimesulide at different scan rates were studied at pH 6.5 by CV (Figure VII (iii a) (p. 232)). The dependence of the peak intensity $I_p$ ($\mu$A) upon the scan rate ($\upsilon$) were carried out to assess whether the process on gold electrode was under diffusion or adsorption-controlled. The influence of the square root of the scan rate on the peak current showed a linear relationship between 10 to 200 mVs$^{-1}$ and the equation can be expressed as follows:

$$I_p = 49.67 \upsilon^{1/2} - 1.303; \ r = 0.9920$$

In addition, there was a linear relation between log $I_p$ and log $\upsilon$, corresponding to the following equation:

$$\log I_p = 0.5183 \log \upsilon + 1.663; \ r = 0.9912$$
Figure VII (i)

Cyclic voltammogram obtained for 1.0 mM nimesulide on gold electrode in pH 6.5, 0.2 M phosphate buffer: (a) blank run (b) nimesulide at \( v = 50 \text{ mV s}^{-1} \)
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Figure VII (ii)

(A) Cyclic voltammogram obtained for 1.0 mM nimesulide in buffer solution at (a) pH 3.0, (b) pH 5.0, (c) pH 6.5, (d) pH 9.2 and (e) pH 11.2 with potential scan rate: 0.05 V s\(^{-1}\) (B) Variation of peak potential with pH for 1.0 mM nimesulide (C) Variation of peak current with pH for 1.0 mM nimesulide
The slope of 0.5183 is close to the theoretically expected value of 0.5 for a purely diffusion controlled process.\textsuperscript{16,17} This indicates that the electrode process was controlled by diffusion rather than adsorption. The peak potential shifted to positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate (Figure VII (iii b) (p. 232)) can be expressed as:

\[ E_p = 0.9979 + 0.0526 \log \nu; \ r = 0.9975 \]

This behavior was consistent with the electrochemical nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step.\textsuperscript{18} As for an irreversible electrode process, according to Laviron,\textsuperscript{19} \( E_p \) is defined by the following equation

\[
E_p = E^0' + \left(\frac{2.303RT}{\alpha nF}\right) \log \left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log \nu \quad (1)
\]

where \( \alpha \) (alpha) is the transfer coefficient, \( k^0 \) the standard heterogeneous rate constant of the reaction, \( n \) the number of electrons transferred, \( \nu \) (nu) the scan rate and \( E^0' \) is the formal redox potential. Other symbols have their usual meanings. Thus, the value of \( \alpha n \) can be easily calculated from the slope of \( E_p \) versus \( \log \nu \). In this system, the slope is 0.0526, then \( \alpha n \) calculated to be 1.1243, taking \( T = 298 \text{ K}, R = 8.314 \text{ JK}^{-1}\text{mol}^{-1} \) and \( F = 96480 \text{ C mol}^{-1} \). For an irreversible process, there is the formula: \( \frac{dE_p}{d\text{pH}} = 0.059X/\alpha n \), where \( X \) is the proton number transferred during the reaction. Accordingly, \( \alpha n = 1.245X \) was
Figure VII (iii)

(A) Cyclic voltammograms of 1.0 mM nimesulide in 0.2 M buffer solution at pH 6.5 at scan rates of: (1) 0.01, (2) 0.03, (3) 0.05, (4) 0.08, (5) 0.1, (6) 0.2 Vs\(^{-1}\)

(B) Plot of variation of peak potential with logarithm of scan rate (y = 0.0526x + 0.9979, R\(^2\) = 0.9951)
obtained. From \(\alpha n = 1.245X\) we got the value of \(X\) to be 1. According to Bard and Faulkner,\(^{20}\) \(\alpha\) can be given as

\[
\alpha = \frac{47.7}{E_p - E_{p/2}} \text{ mV}
\]

where \(E_{p/2}\) is the potential where the current is at half the peak value. So, from this we got the value of \(\alpha\) to be 0.5. Further, the number of electron (\(n\)) transferred in the electro oxidation of nimesulide was calculated to be 2.2 \(\approx 2\). Thus, it may assume that the electrode reaction of nimesulide was accompanied by one proton and two electrons, i.e., nitrogen atom in methanesulfonamide group of nimesulide loses one proton and two electrons and forms the final oxidized product. This was also supported by the earlier report.\(^{21}\) The value of \(k_0\) can be determined from the intercept of the above plot if the value of \(E^{0'}\) is known. The value of \(E^{0'}\) in equation (1) can be obtained from the intercept of \(E_p\) versus \(\nu\) curve by extrapolating to the vertical axis at \(\nu = 0\).\(^{22}\) In our system the intercept for \(E_p\) versus \(\log \nu\) plot was 0.9979 and \(E^{0'}\) was obtained to be 0.9464, the \(k_0\) was calculated to be 287.1 s\(^{-1}\).

### 7.3.5. Mechanism

Nimesulide showed one well-resolved anodic signal in all pH range studied. It is found to exist in two different forms, ionized and non-ionized, due to the pKa of nimesulide as 6.5. Consequently, near pH 6.5 we can anticipate to obtain the two forms according to the equilibrium shown in Scheme 2.
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Scheme 2

In acid media the oxidation of nimesulide follows a proton-dependent mechanism while in alkaline media protons are not involved in the rate determining step or before. In the acid range an increase of the peak current with the increase of pH was observed. On the other hand, in the basic range decrease in the peak current with the increase of pH was observed. The anodic peak could be attributed probably to the methylsulfonamide group oxidation contained in the structure of nimesulide. Since, \( N \) in methanesulfonamide is
basic, acidic condition is required for the oxidation which is facilitated by pH = 6.5 in this study, thus showing high peak current at pH = 6.5. Based on all these observations we postulated the mechanism as shown in Scheme 2.

7.3.6. Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the DPV mode, because the peaks are sharper and better defined at lower concentration of nimesulide than those obtained by CV, with a lower background current, resulting in improved resolution. The analytical characteristics of the calibration plot are summarized in Table VII (i) (p. 237). According to the obtained results, it was possible to apply this technique to the quantitative analysis of nimesulide. The PBS of pH 6.5 was selected as the supporting electrolyte for the quantification as nimesulide gave maximum peak current at pH 6.5. Differential pulse voltammograms obtained with increasing amounts of nimesulide showed that the peak current increased linearly with increasing concentration, as shown in Figure VII (iv) (p. 238). It was found that the plot of $I_p$ versus concentration showed linearity over the drug concentration range of $2.0 \times 10^{-7}$ to $1.2 \times 10^{-6}$ M. The linear equation was $I_p (\mu A) = 0.75 + 3.229 C \ (r = 0.9961, C \text{ is in } \mu \text{M})$. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation products of nimesulide on the electrode surface.\textsuperscript{23} Related statistical data of the calibration curves were obtained from the five different calibration curves. Limit of
detection (LOD) and quantification (LOQ) were calculated based on the peak current using the following equations shown below.

\[ \text{LOD} = 3 \frac{s}{m}; \quad \text{LOQ} = 10 \frac{s}{m}. \]

where \( s \) is the standard deviation of the peak currents of the blank (five replicates), and \( m \) is the slope of the calibration curve. The LOD and LOQ values were calculated to be \( 1.11 \times 10^{-9} \) M and \( 3.70 \times 10^{-9} \) M, respectively. The LOD and LOQ values calculated by the present method are better compared to the reported work. The detection limits reported at different electrodes are tabulated in Table VII (ii) (p. 237). Analyzing five replicates, for the process of the validation within-day variations and for intraday assay were studied. The corresponding percentage of RSD values (Table VII (i) (p. 237)) indicates good repeatability and reproducibility.

### 7.3.7. Tablet analysis

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, a commercial medicinal sample containing nimesulide i.e., Nicip Plus (100 mg per tablet) was used. The tablets were grounded to powder, dissolved in methanol and then further diluted so that nimesulide concentration falls in the range of calibration plot. The contents of the flask were sonicated for 10 min to affect complete dissolution. Differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential pulse voltammograms for
### Table VII (i)
Characteristics of nimesulide calibration plot using differential pulse voltammetry at gold electrode

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (M)</td>
<td>$2.0 \times 10^{-7}$ to $1.2 \times 10^{-6}$</td>
</tr>
<tr>
<td>Slope of the calibration plot ($\mu$ A M$^{-1}$)</td>
<td>$3.25 \times 10^{-6}$</td>
</tr>
<tr>
<td>Intercept ($\mu$ A)</td>
<td>0.75</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9961</td>
</tr>
<tr>
<td>RSD of slope (%)</td>
<td>1.37</td>
</tr>
<tr>
<td>RSD of intercept (%)</td>
<td>1.63</td>
</tr>
<tr>
<td>Number of data points</td>
<td>6</td>
</tr>
<tr>
<td>LOD (M)</td>
<td>$1.11 \times 10^{-9}$</td>
</tr>
<tr>
<td>LOQ (M)</td>
<td>$3.70 \times 10^{-9}$</td>
</tr>
<tr>
<td>Repeatability (RSD %)</td>
<td>1.16</td>
</tr>
<tr>
<td>Reproducibility (RSD %)</td>
<td>1.27</td>
</tr>
</tbody>
</table>

### Table VII (ii)
Comparison of detection limits of nimesulide by different methods

<table>
<thead>
<tr>
<th>Different methods</th>
<th>Detection limits (M)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometric determination</td>
<td>$4.5 \times 10^{-8}$</td>
<td>7</td>
</tr>
<tr>
<td>Glassy carbon electrode modified by cysteic acid/CNTs</td>
<td>$5.0 \times 10^{-8}$</td>
<td>4</td>
</tr>
<tr>
<td>Multiwalled carbon nanotubes modified glassy carbon electrodes</td>
<td>$1.6 \times 10^{-7}$</td>
<td>5</td>
</tr>
<tr>
<td>Electro-oxidation on gold electrode</td>
<td>$1.1 \times 10^{-9}$</td>
<td>Present work</td>
</tr>
</tbody>
</table>
Figure VII (iv)

Differential pulse voltammograms with increasing concentration of nimesulide in pH 6.5, 0.2 M phosphate buffer solution on gold electrode with nimesulide concentration: (a) blank, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.0, (g) 1.2 µM, Inset: plot of current vs. concentration of nimesulide
plotting calibration plot. The results are in good agreement with the content marked in the label.

The recovery test of nimesulide ranging from $1.0 \times 10^{-5}$ to $1.0 \times 10^{-4}$ M was performed using differential pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulations of nimesulide. The F and Student t tests were also calculated. All these are results are listed in Table VII (iii) (p. 240). The recoveries in different samples were found to lie in the range from 97.3 to 100.2 %, with RSD of 1.41%.

For the possible analytical application of the proposed method, the effect of some common excipients used in pharmaceutical preparations was examined. Differential pulse voltammetric experiments were carried out for $1.0 \times 10^{-5}$ M nimesulide in the presence of 1.0 mM of each of the excipients. It was observed that 100 folds of citric acid, dextrose, glucose, gum acacia, lactic acid, oxalic acid, starch and sucrose did not interfere with the voltammetric signal of nimesulide. Thus, the procedures were able to assay nimesulide in the presence of excipients, and hence it can be considered specific.

7.3.8. Detection of TP in urine samples

The applicability of the proposed method for the determination of nimesulide in biological fluid of human urine was attempted. Drug-free human and urine samples, obtained from healthy volunteers, filtered through a filter
Table VII (iii)

Analysis of nimesulide in tablets by DPV and recovery studies

<table>
<thead>
<tr>
<th></th>
<th>Nicip plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled claim (mg)</td>
<td>100</td>
</tr>
<tr>
<td>Amount found (mg)*</td>
<td>98.5</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.41</td>
</tr>
<tr>
<td>*-test of significant</td>
<td>0.49</td>
</tr>
<tr>
<td>*-test of significant</td>
<td>1.02</td>
</tr>
<tr>
<td>Bias (%)</td>
<td>-2.5</td>
</tr>
<tr>
<td>Added (mg)</td>
<td>1.0</td>
</tr>
<tr>
<td>Found (mg)*</td>
<td>0.98</td>
</tr>
<tr>
<td>Recovered (%)</td>
<td>98.4</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.33</td>
</tr>
<tr>
<td>Bias (%)</td>
<td>-2.0</td>
</tr>
</tbody>
</table>

*Average of five determinations

Table VII (iv)

Application of DPV to the determination of nimesulide in spiked human urine samples

<table>
<thead>
<tr>
<th>Urine Samples</th>
<th>RSD (%)</th>
<th>Bias (%)</th>
<th>Spiked (10^-6 M)</th>
<th>Found (10^-6 M)*</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.69</td>
<td>-2.0</td>
<td>0.1</td>
<td>0.098</td>
<td>98.0</td>
</tr>
<tr>
<td>2</td>
<td>0.48</td>
<td>-0.5</td>
<td>0.2</td>
<td>0.199</td>
<td>99.5</td>
</tr>
<tr>
<td>3</td>
<td>1.40</td>
<td>-1.6</td>
<td>0.5</td>
<td>0.492</td>
<td>98.4</td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
<td>-1.3</td>
<td>0.8</td>
<td>0.790</td>
<td>98.8</td>
</tr>
<tr>
<td>5</td>
<td>1.08</td>
<td>-1.1</td>
<td>1.0</td>
<td>0.989</td>
<td>98.9</td>
</tr>
</tbody>
</table>

*Average of five determinations
paper and stored frozen until the assay was performed. The developed differential pulse voltammetric method for the nimesulide determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of nimesulide. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of nimesulide into the detect system of urine sample. The calibration graph was used for the determination of spiked nimesulide in urine samples. The detection results of four urine samples obtained are listed in Table VII (iv) (p. 240). The recovery determined was in the range from 97.9 to 100.5 % and the RSD was 1.03 %. Good recoveries of nimesulide were achieved from these matrices, denoting that application of the proposed method to the analysis of nimesulide in biological fluid could be easily assessed.

7.4. IMPORTANCE OF CHAPTER VII

The electrochemical behavior of nimesulide at gold electrode surface was investigated by cyclic voltammetry in phosphate buffer solution (pH = 6.5). Based on the study, influence of several physico-chemical parameters like potential scan rate, pH and concentration were investigated. The oxidation of nimesulide was found to be an irreversible, two electron-one proton process and is a diffusion controlled process. The peak current was linear to nimesulide concentrations over a certain range, under the selected conditions. This helps in
voltammetric determination of selected analyte with lower concentrations and can be used successfully to assay the drug in pharmaceutical dosage form as well as in spiked urine samples. High percentage recovery and study of excipients showed that the method is free from the interferences of the commonly used excipients and additives in the formulations of drugs. In addition, the results obtained in the analysis of nimesulide in spiked urine samples demonstrated the applicability of the method in real sample clinical analysis. The proposed method is suitable for quality control laboratories as well as pharmacokinetic studies with satisfactory results.
7.5. REFERENCES

1. A. Bernareggi,
   Inflammopharmacology, 9, 81 (2001)

2. A. Maltese, F. Maugeri and C. Bucolo,

3. C. K. Zacharis, P. D. Tzanavaras, M. Notou, A. Zotou and D. G. Themelis,

4. C. Y. Wang, X. Q. Shao, Q. X. Liu, Q. S. Qu, G. J. Yang and X. Y. Hu,

5. J. Zhang, X. Tan, D. Zhao, S. Tan, Z. Huang, Y. Mi and Z. Huang,

6. B. Hemmateenejad, K. Javidnia and M. Saeide-Boroujeni,

7. I. C. Constantinescu, M. Florea, C. C. Arama, A. Nedelcu and C. M. Monciu,
   Farmacia, 57, 267 (2009)

8. S. Furlanetto, S. Orlandini, G. Aldini, R. Gotti, E. Dreassi and S. Pinzauti,


10. E. E. Ferapontova,

11. H. Y. Xia and X. Y. Hu,

12. G. D. Christian and W. C. Purdy,


