SENSITIVE SPECTROPHOTOMETRIC DETERMINATION OF
TRAMADOL HYDROCHLORIDE IN PHARMACEUTICALS
USING FOLIN-CIOCALTEU’S REAGENT

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Abstract
A simple, rapid and sensitive method is described for the determination of tramadol hydrochloride (TMH) in pure form or in tablets. The method is based on the reduction of Folin-Ciocalteu (F-C) reagent by TMH in alkaline medium, leading to the formation of a blue coloured chromogen, which absorbs maximally at 760 nm. Under the optimized experimental conditions, the colour is stable for over 12 h and Beer’s law is obeyed in the concentration range of 2.5-50 µg/mL. The regression coefficient (r) is calculated to be 0.9998 with molar absorptivity value of $6.3 \times 10^3$ L/mol/cm and Sandell sensitivity of 0.0478 µg/cm per 0.001 absorbance unit. The limits of detection (LOD) and quantification (LOQ) are also reported. Within-day variation determined on three different concentrations showed accuracies ranging from 0.70 to 1.53% and the RSD was determined to be < 2%. Day to day variation presented accuracies ranging from 1.12 to 1.53% with an RSD < 2%. The proposed method was successfully applied to the determination of TMH in tablets with good recoveries.

Key words: Tramadol, Determination, F-C reagent, Spectrophotometry, Pharmaceuticals.

Tramadol Hidroklori̇rin Folin-Ciocalteu Reaktifi Kullanılarak Farmasötik
Preparatlarda Hassas Olarak Spektrofotometrik Tayini

Tramadol HCl’in (TMH) saf halde veya tablet formundaki miktar tayini için basit, hızlı ve hassas bir yöntem tanımlanmıştır. Yöntem, alkali ortamda TMH ile Folin-Ciocalteu (F-C) reaktifinin indirgenmesini temel alan 760 nm’dde maksimum absorbsiyon veren mavi renkli rengeli rengeleninen dayanmaktadır. Optimized edilmiş deneySEL koşullar altında, renk 12 saat boyunca stabildir ve 2.5-50 µg/mL konsantrasyon aralığında Beer kanununa uymaktadır. Regresyon katsayısı 0.9998, molar absorbptivite değeri $6.3 \times 10^3$ L/mol/cm ve Sandell hassasiyeti 0.0478 µg/cm/0.001 absorbans ünitesi olarak hesaplanmıştır. Deteksiyon limiti (LOD) ve miktar tayini limiti (LOQ) belirtilmiştir. Üç konsantrasyon üzerinden hesaplanan gün-iç varyasyon değeri % 0.70-1.53 aralığında doğrudan BSS değeri %2’ nin altında olarak hesaplanmıştır. Günler-arası varyasyon değeri %1.12-1.53 aralığında kesinlik göstermiş ve BSS %2’ nin altında bulunmaktadır. Önerilen metod, TMH’in tabletlerinden miktar tayini için iyi bir geri kazanım ile başarılı bir şekilde uygulanmıştır.

Anahtar kelimeler: Tramadol, Miktar tayini, F-C reaktifi, Spektrofotometri, Farmasötik preparatlar

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INTRODUCTION

Tramadol hydrochloride (TMH), chemically known as (1R, 2R)-rel-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol; (±)-cis-2 (Figure 1), is a synthetic analogue of codeine. It is a centrally acting analgesic agent (1) and has been used since 1977 for relief of severe physical pain and has been the most widely sold opioid analgesic drug in the world (2).

Figure 1. Structure of TMH.

Because TMH is widely used, several methods have been employed to determine TMH in pharmaceutical formulations and they include high-performance liquid chromatography (3-6), thin-layer chromatography-densitometry (7), capillary isotachophoresis (8), ion-selective electrode based potentiometry (9-15), adsorptive stripping voltammetry (16), square-wave voltammetry (17), UV-spectrophotometry (3,18,19), and flow injection chemiluminescence spectrophotometry (20). The above methods are deficient on simplicity, cost-effectiveness and easy accessibility.

Visible spectrophotometry, despite its speed, sensitivity, fair selectivity, high accuracy and precision, has been sparsely employed in the assay of TMH in pharmaceuticals. Abdellatef et al. (21) have reported a method based on the condensation of TMH with the mixed hydrides of malonic and acetic acids at 60°C for 40 min. Though the method looks sensitive, it is applicable over a narrow range of 0.5-2.5 µg/mL for TMH. Also, the measurement is made at 330 nm where the interference from the tablet excipients is far more than at longer wavelengths. One of the same authors (22) has described the assay of either TMH by two kinetic spectrophotometric procedures based on the kinetic investigation of either the oxidation of TMH with alkaline KMnO₄ at room temperature for a fixed time of 20 min where the absorbance of the coloured manganate was measured at 610 nm or the reaction of TMH with 4-chloro-7-benzofurazan (NBD-Cl) in the presence of 0.1M Na₂CO₃. In this procedure, the spectrophotometric measurements were recorded by measuring the absorbance at 467 nm for a fixed time of 25 min in a thermostated water bath maintained at 90 ± 1°C. The procedures are reported to be applicable over 5-25 µg/mL (using KMnO₄) and 50-250 µg/mL (using NBD-Cl) TMH. These procedures, apart from being applicable over narrow linear dynamic ranges require judicious control of all experimental variables. Extractive spectrophotometric method based on the formation of ion-pair complex with bromocresol green in acidic medium followed by extraction into CH₂Cl₂ and absorbance measurement at 417 nm has been reported by Rajput and Trivedi (23) for the determination of TMH in capsule and injection formulations. The method is applicable over 2-15 µg/mL TMH with ε value of 9.92 × 10³ l/mol/cm. In the same article, a second procedure based on the reaction of TMH with Folin-Ciocalteu (F-C) reagent in NaOH medium is also described where the absorbance of the coloured product is measured at 645 nm. The method has a linear range of 10-40 µg/mL and ε value of 3.03 × 10³ l/mol/cm. But, to the best of our knowledge, the reduction product of F-C reagent, a blue coloured chromogen absorbs maximally at 760 nm (24-33) in alkaline medium which the authors have failed to observe in their investigation. This prompted us to reinvestigate this reaction and the result is a
simple and rapid spectrophotometric method based on the redox reaction between TMH and F-C reagent in Na\textsubscript{2}CO\textsubscript{3} medium, the resulting blue coloured species being measured at 760 nm. This modification has some advantages over the previously reported method using the same reagent, the greatest of which are high sensitivity and wide linear dynamic range. The method has the requisite accuracy, precision, and selectivity to determine TMH in tablets.

**EXPERIMENTAL**

**Instrument**

A Systronic model 106 digital spectrophotometer (Ahmed Abad, India) with matched 1-cm quartz cells was used for absorbance measurements.

**Reagents and Standards**

Chemicals used were of analytical grade. Distilled water was used throughout the investigation. Folin-Ciocalteu reagent (Merck, Mumbai, India), sodium carbonate (S. D. Fine Chem. Ltd., Mumbai, India) used were of analytical reagent grade and used without further purification. Pure TMH (Pharmaceutical grade) sample was kindly provided by Jubilant Organosys Ltd., Nanjangud, Mysore, India, as a gift and used as received. Four brands of tablets, namely, Tramazac-TC 100 (Zydus Alidac Pvt. Ltd.), Contramol-DT (Piramal healthcare) and Cemadol 50 CR (Life Medicare & Biotech Pvt. Ltd.), Trambax 50 Oro-Dispersible(Ethypharm LL Pvt. Ltd., Amberth(w)Thane) were obtained from the commercial sources.

**Standard drug solution**

A stock standard solution of TMH (1000 µg/mL) was prepared by dissolving 50 mg of pure TMH in water and making the volume to 50 mL in a volumetric flask. Working concentration of TMH (100 µg/mL) was prepared by further dilution of the above stock solution with water.

**F-C Reagent (v/v)**

A 1: 1 aqueous solution was prepared by mixing accurately measured 50 mL of F-C reagent with 50 mL of water.

**Sodium carbonate (Na\textsubscript{2}CO\textsubscript{3})**

A fifteen percent (15% w/v) solution was prepared by dissolving 15 g of the pure sodium carbonate in 100 mL of water, and filtered.

**Preparation of Calibration Graph**

Different aliquots of working standard TMH solution (100µg/mL) ranging from 0.25-5 mL were transferred into a series of 10 mL of volumetric flasks using a micro burette and the total volume was brought to 5 mL with water. To each flask, 1 mL of 1:1 F-C reagent and 2 mL of 15% Na\textsubscript{2}CO\textsubscript{3} solution were successively added by means of a micro burette. The flasks were stoppered, content mixed well and kept to room temperature for 15 min. The volume was made up to the mark with water and the absorbance of each solution was measured at 760 nm against a reagent blank similarly prepared in the absence of TMH.

Standard graph was prepared by plotting the absorbance *versus* drug concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using the absorbance-concentration data.

**Procedure for Tablets**

An amount of finely ground tablet powder equivalent to 10 mg of TMH was accurately weighed into a 100-mL volumetric flask, the flask was shaken with ~70 mL of water for about
20 min; and finally volume was made up to the mark with water. The flask was kept aside for 5 min, and the mixture was filtered using Whatman No. 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot (say 2-3 mL) was used for assay as described earlier.

RESULTS AND DISCUSSION

Folin-Ciocalteu’s reagent (F-C) is specially used for the determination of many phenolic compounds utilizing its liability to be reduced into blue colored product. Many drug substances such as salbutamol (24), minocycline (25), diclofenac (26), trimetazidine (27), acyclovir (28), methotrexate (29), omeprazole (30), sulphipyrazone (31), and gliclazide (32) have been determined on this basis. The structural features of TMH allowed the use of F-C reagent for its assay. The proposed method is based on the formation of a blue colored chromogen, following the reduction of phospho-molybdo tungstic mixed acid of the F-C reagent (33) by TMH, in the presence of sodium carbonate, which could be measured at 760 nm. The mixed acids in the F-C reagent are the final chromogen and involve the following chemical species:

\[
\begin{align*}
&3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O \\
&3H_2O \cdot P_2O_5 \cdot 14WO_3 \cdot 4MoO_3 \cdot 10H_2O
\end{align*}
\]

TMH probably effects reduction of oxygen atoms from tungstate and/or molybdate in the F-C reagent, there by producing one or more possible reduced species which have characteristic intense blue colour.

Absorption Spectra

TMH reacts with F-C reagent in the presence of Na$_2$CO$_3$ to form intensely blue coloured product with an absorption maximum at 760 nm. Figure 2 shows the absorption spectra of the reaction product and reagent blank. The coloured product showed a maximum absorbance at 760 nm, which was used as the wavelength for determination. Under the same experimental conditions the blank had negligible absorbance.

Method Development

Optimization of experimental variables

A series of preliminary experiments necessary for rapid and quantitative formation of colored product to achieve the maximum stability and sensitivity was performed. Optimum condition was fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 760 nm.

Selection of reaction medium and optimization of the base

To find a suitable medium for the reaction, different aqueous bases such as sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate and sodium hydrogen phosphate were investigated. Better results obtained with sodium carbonate. In order to determine the optimum concentration of Na$_2$CO$_3$, different volumes of 15% Na$_2$CO$_3$ solution (1 – 4 mL) were attempted at a constant concentration of TMH (20 μg/mL) and the results of the observation are
showed in Figure 3. It was found that maximum and constant absorbance values were obtained at 2 to 4 mL of 15% Na$_2$CO$_3$ thus 2.0 mL was used throughout the work.

![Figure 3](image)

**Figure 3.** Effect of Na$_2$CO$_3$ concentration on the absorbance value of the coloured product.

*Effect of the F-C reagent concentration*

Several experiments were carried out to study the influence of F–C reagent concentration on the color development and the obtained results are shown in Figure 4. It is apparent that 1.0 to 2.0 mL of reagent gave the maximum color intensity, thus 1.0 mL of reagent was used throughout the investigation.

![Figure 4](image)

**Figure 4.** Effect of different volumes of F–C reagent (1:1) on the reaction product with TMH (20 µg/mL) in Na$_2$CO$_3$ solution.

*Effect of reaction time and stability of the colour*

Colour formation was not instantaneous. Maximum color development was obtained in 15 min after mixing the reactants, and the colour was stable for at least 24 hours thereafter.

*Effect of order of addition of reactants*

The sequence of order of addition of the reactants had significant effect on the absorbance value. So, the order used in the general procedure should be followed for maximum absorbance.

*Method Validation*

The proposed method has been validated for Linearity, sensitivity, limits of detection and quantification, precision, accuracy, selectivity and recovery.
Linearity and sensitivity

A linear correlation was found between absorbance at $\lambda_{\text{max}}$ and concentration of TMH in the range given in Table 1. Regression analysis of the Beer’s law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. A plot of log absorbance and log concentration, yielded straight line with slope equal to 1.013 further establishing the linear relation between the two variables. The optical characteristics such as Beer’s law limits, molar absorptivity and Sandell sensitivity values (34) of the method are also given in Table 1.

Table 1. Sensitivity and regression parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}, \text{nm}$</td>
<td>760</td>
</tr>
<tr>
<td>Linear range, $\mu$g/mL</td>
<td>2.5-50</td>
</tr>
<tr>
<td>Molar absorptivity ($\varepsilon$), L/mol/cm</td>
<td>$6.3 \times 10^3$</td>
</tr>
<tr>
<td>Sandell sensitivity*, $\mu$g/cm</td>
<td>0.0478</td>
</tr>
<tr>
<td>Limit of detection (LOD), $\mu$g/mL</td>
<td>0.67</td>
</tr>
<tr>
<td>Limit of quantification (LOQ), $\mu$g/mL</td>
<td>2.04</td>
</tr>
<tr>
<td>Regression equation, $Y**$</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.0087</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0216</td>
</tr>
<tr>
<td>Standard deviation of a ($S_a$)</td>
<td>0.3675</td>
</tr>
<tr>
<td>Standard deviation of b ($S_b$)</td>
<td>0.0074</td>
</tr>
<tr>
<td>Variance ($S_{a}^2$)</td>
<td>0.1351</td>
</tr>
<tr>
<td>Regression coefficient (r)</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

$^*$Limit of determination as the weight in $\mu$g per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm$^2$ and $l = 1$ cm; $^**Y = a + bX$, Where Y is the absorbance; X is concentration in $\mu$g/mL; a intercept and b slope.

The limits of detection (LOD) and quantification (LOQ) calculated according to ICH guidelines (35) using the formulae: LOD = 3.3 S/b and LOQ = 10 S/b, (where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot) are also presented in Table 1. The high value of $\varepsilon$ and low value of Sandell sensitivity and LOD indicate the high sensitivity of the proposed method.

Precision and accuracy

The assay described under “general procedure” was repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the method. The assay was performed for three levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤ 2% (intra-day and inter-day) indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and taken concentrations for TMH. Bias (bias % = [(Concentration found - known concentration) x 100 / known concentration]) was calculated at
each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ≤ 2% demonstrate the high accuracy of the proposed method.

### Table 2. Evaluation of intra-day and inter-day accuracy and precision.

<table>
<thead>
<tr>
<th>TMH taken, µg/mL</th>
<th>Intra-day accuracy and precision (n=7)</th>
<th>Inter-day accuracy and precision (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMH found, µg/mL</td>
<td>%RE</td>
</tr>
<tr>
<td>10</td>
<td>10.07</td>
<td>0.70</td>
</tr>
<tr>
<td>20</td>
<td>19.79</td>
<td>1.05</td>
</tr>
<tr>
<td>30</td>
<td>29.59</td>
<td>1.37</td>
</tr>
</tbody>
</table>

%RE= Percent relative error, %RSD= Relative standard deviation.

**Selectivity**

A systematic study was performed to determine the effect of matrix by analyzing the placebo blank and synthetic mixture containing TMH. A placebo blank of the composition: starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under ‘tablets’, and then subjected to analysis. The absorbance of the placebo solution was almost equal to the absorbance of the blank which revealed no interference. To assess the role of the inactive ingredients on the assay of TMH, a synthetic mixture was separately prepared by adding 10 mg of TMH to the placebo mentioned above. The drug was extracted and the solution (100 µg/mL) was prepared as described under the general procedure for tablets. The solution was analyzed following the recommended procedure. The absorbances resulting from 20, 30 and 40 µg/mL TMH solution were nearly the same as those obtained for pure TMH solutions of identical concentrations. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of TMH. Further, the slope of the calibration plot prepared from the synthetic mixture solutions was about the same as that prepared from pure drug solution. Reducing ions, tryptophan, hydroxyproline, 2- and 3-hydroxypyridines, ascorbic acid and uric acid also reduce F-C reagent to molybdenum blue (36). However, these substances are seldom present in the reagents used and in the tablets employed. Hence, the method is devoid of error due to above substances.

**Robustness**

The robustness of the method was evaluated by making small incremental changes in the volume of F-C reagent/Na₂CO₃ and reaction time, and the effect of the changes was studied by calculating the mean RSD values. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (≤ 3%).

**Ruggedness**

Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using four different instruments. The inter-analysts RSD were within 2% whereas the inter-instruments RSD for the same TMH amount was less than about 4% suggesting that the developed method was rugged. The results are shown in Table 3.
Table 3. Method robustness and ruggedness expressed as intermediate precision (% RSD).

<table>
<thead>
<tr>
<th>TMH taken, µg/mL</th>
<th>Robustness</th>
<th>Ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter altered</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volume of Na₂CO₃ (%RSD)</td>
<td>Reaction time* (%RSD)</td>
</tr>
<tr>
<td>10</td>
<td>1.67</td>
<td>1.26</td>
</tr>
<tr>
<td>20</td>
<td>1.85</td>
<td>1.78</td>
</tr>
<tr>
<td>30</td>
<td>1.97</td>
<td>2.04</td>
</tr>
</tbody>
</table>

*Volumes of Na₂CO₃ added were 1.5, 2.0 and 2.5 mL.
*The reaction times studied were 13, 15 and 17 min.

Application to formulations

The described procedure was successfully applied to the determination of TMH in its tablet formulation (Tramazac –TC 100, Contramol-DT and Cemadol 50 CR). The results obtained (Table 4) were statistically compared with the reference UV method. (19). The method consisted of the measurement of the absorbance of tablet extract in water at 271 nm. The results obtained by the proposed method agreed well with those of reference method and with the label claim. The results were also compared statistically by a Student’s t-test for accuracy and by a variance F-test for precision with those of the reference method at 95 % confidence level as summarized in Table 4. The results showed that the calculated t-and F-values did not exceed the tabulated values inferring that proposed method is as accurate and precise as the reference method.

Recovery studies

To further assess the accuracy of the method, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure TMH at three different levels (50, 100 and 150 % of the content present in the tablet powder (taken) and the total was found by the proposed method. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 99.85 and 105.6% with relative standard deviation in the range 0.76-1.26%. Closeness of the results to 100% showed the fairly good accuracy of the method. The results are shown in Table 5.
Table 4. Results obtained by the analysis of tablets by the proposed methods and statistical comparison of results with the reference method.

<table>
<thead>
<tr>
<th>Tablet brand name</th>
<th>Nominal amount, (mg/tablet)</th>
<th>Found* (Percent of label claim ± SD)</th>
<th>Reference method</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tramazac</td>
<td>100</td>
<td>101.3 ± 0.58</td>
<td>102.4 ± 0.96</td>
<td>( t = 1.64 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( F = 2.73 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98.76 ± 0.72</td>
<td></td>
<td>100.1 ± 1.12</td>
</tr>
<tr>
<td>Trambax</td>
<td>50</td>
<td>103.5 ± 0.64</td>
<td>104.6 ± 0.82</td>
<td>( t = 2.30 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( F = 2.41 )</td>
</tr>
<tr>
<td>Cemadol</td>
<td>100</td>
<td>102.4 ± 0.96</td>
<td>102.4 ± 0.96</td>
<td>( F = 1.64 )</td>
</tr>
</tbody>
</table>

*Average of five determinations.
Tabulated \( t \) value at the 95% confidence level is 2.77. Tabulated \( F \) value at the 95% confidence level is 6.39.

Table 5. Results of recovery study using standard addition method.

<table>
<thead>
<tr>
<th>Tablet studied</th>
<th>TMH in tablet extract, ( \mu g/mL )</th>
<th>Pure TMH added, ( \mu g/mL )</th>
<th>Total TMH found, ( \mu g/mL )</th>
<th>Pure TMH recovered (Percent±SD*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tramazac</td>
<td>10.24</td>
<td>5.0</td>
<td>5.39</td>
<td>105.6 ± 0.76</td>
</tr>
<tr>
<td>100mg</td>
<td>10.24</td>
<td>10.0</td>
<td>10.43</td>
<td>103.3 ± 1.11</td>
</tr>
<tr>
<td></td>
<td>10.24</td>
<td>15.0</td>
<td>15.29</td>
<td>101.2 ± 0.82</td>
</tr>
<tr>
<td>Trambax</td>
<td>10.01</td>
<td>5.0</td>
<td>5.10</td>
<td>99.85 ± 0.92</td>
</tr>
<tr>
<td>50mg</td>
<td>10.01</td>
<td>10.0</td>
<td>10.14</td>
<td>100.4 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>10.01</td>
<td>15.0</td>
<td>15.36</td>
<td>101.7 ± 1.26</td>
</tr>
</tbody>
</table>

*Mean value of three determinations.
CONCLUSIONS

The important features of the modified method using F-C reagent are simplicity, speed, cost-effectiveness and easy accessibility to the instrument employed which is inexpensive compared to most techniques reported for tramadol. The method is superior to all the reported spectrophotometric methods in terms of sensitivity (ε = 6.3×10^3) and linear dynamic range (2.5-50.0 µg/mL). Further, in the reference method, the absorbance is measured at 271 nm where the interference from excipients particularly with unsaturation is very serious and the present methods are free from such interference. Moreover, the present method is rapid with respect to analysis time and directly applicable to the drug sample without prior extraction into organic solvents needed in other techniques. Further, the method is free from critical optimum conditions such as heating or extraction step, pH control or use of toxic organic solvents. The method has good linearity, high analytical accuracy and precision. These characteristics together with low detection limit and absence of matrix effect make the method suitable for use in pharmaceutical quality control laboratories.

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