Research Article

Stripping Voltammetric Determination of Analgesics in Their Pharmaceuticals Using Nano-Riboflavin-Modified Glassy Carbon Electrode

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Cyclic voltammetric behaviors of three analgesics, acetaminophen (AAP), acetylsalicylic acid (ASA), and dipyrone (DP), were studied using nano-riboflavin-modified glassy carbon electrode. One well-defined oxidation peak each for AAP and ASA and three oxidation peaks for DP were observed. The influence of pH, scan rate, and concentration reveals irreversible diffusion controlled reaction. The SEM analysis confirmed good accumulation of the drugs on the electrode surface. Calibration was made under the maximum peak current conditions. The concentration range studied for the determination of drugs was 0.02 to 0.4 \( \mu g \) mL\(^{-1}\) for AAP and ASA and 0.025 to 0.4 \( \mu g \) mL\(^{-1}\) for DP. The lower limit of detection observed for AAP, ASA, and DP was 0.016, 0.007 \( \mu g \) mL\(^{-1}\), and 0.013 \( \mu g \) mL\(^{-1}\), respectively. The suitability of the method for the determination of these analgesics in pharmaceutical preparations and urine samples was also ascertained.

1. Introduction

Stripping voltammetry is one of the most important and significant electroanalytical methods to determine the trace level pharmaceutical samples. By employing anodic/cathodic/adsorptive-stripping voltammetry, \( \mu g \) mL to ng/mL range of drugs can be determined. Sensitivity of the method can be increased by carrying out the stripping using differential pulse or square wave mode.

In recent years, numerous investigations have been carried out on electrochemical biosensors to improve the rapidity, selectivity, and sensitivity and to reduce the cost of analysis [1]. The understanding of the versatile chemistry of flavins is of extreme importance and has been the focus of many investigations [2]. The flavins have in common the isalloxazine ring system, where the redox process occurs. The redox process of the flavins is thermodynamically reversible, irrespective of whether one or two electrons per flavin molecule are being transferred [3]. Many electrochemical investigations have been made with the goal of elucidating the mechanism and using flavins as electrode modifier. Surface or bulk modified carbon paste electrodes with flavins are useful configurations to incorporate electroactive species for analytical purposes [4].

The riboflavin (RF), vitamin B2, is an essential precursor of flavin adenine dinucleotide and flavin mononucleotide coenzymes. The chemical environment around riboflavin modulates its redox behaviour by enhancing or preventing the targeted redox reaction. The best electrocatalytical activity was found to be the mixed system composed of riboflavin and 3,3'-thiodipropionic acid [5]. A stable, novel Nafion-riboflavin functional membrane modified gold electrode was constructed and used for the study on the electron transfer of cytochrome C, superoxide dismutase, and hemoglobin [6]. A chemically modified electrode exhibiting electrocatalytic response towards dioxygen was constructed by adsorbing
the mediator riboflavin onto spectroscopic graphite [7]. The adsorbed riboflavin as an effective mediator can accelerate the reduction of dioxygen, which accepts two electrons from the reduced riboflavin to generate hydrogen peroxide. The rate constants of the electrocatalytic reaction in various pH solutions were determined using a rotating disc electrode modified with riboflavin [8–10]. The electrochemical behavior of riboflavin (RF) adsorbed on different surfaces of inorganic matrices was investigated using modified carbon paste electrodes. The immobilization of RF on these materials indicated high electrode stability avoiding leaching out of the electroactive species (RF) from the electrode surface [11]. An original electroenzymatic system, reductase-riboflavin, was employed for the improvement of dehydrogenase-based biosensors in the amperometric detection of lactate [12]. However, the development of RF-modified electrode for the electroanalysis of drugs has not been given much attention. Hence it is thought of developing nano-riboflavin-modified electrode for the determination of certain common drugs.

Acetaminophen (AAP) is a popular analgesic and antipyretic agent [13]. Many assays have been determined using titrimetry, chromatography, fluorometry, colorimetry, spectrophotometry, electrochemical, and pulse perturbation techniques [14–18]. Electrochemical studies of AAP using various electrodes such as conducting polymer-modified electrode [19], sodium montmorillonite clay-modified electrode [20], nanopolypyrrole-modified glassy carbon electrode [21], pumice mixed carbon electrodes [22], C60-modified glassy carbon electrode [23], PANI-MWCNTs composite-modified electrode [24], single-wall carbon nanotube-dicetyl phosphate film [25], and nickel magnetic nanoparticles-modified electrodes [26] were reported.

Acetylsalicylic acid (ASA) possesses anti-inflammatory, analgesic and antipyretic properties. Partial least-squares algorithm, reversed-phase sequential injection chromatography technique, ultraviolet, fluorescence, infrared methods, chromatographic procedures (HPLC), and atomic absorption spectrometry have been described for the determination of ASA [27, 28]. These methods are not frequently used because of tedious sample preparations involved, expensive apparatus and usually needed, extraction procedures. Ion selective electrodes have been developed showing high specificity, good detection limits, and relatively low cost [29]. The electrochemical oxidation of salicylic acid in pharmaceuticals formulations of ASA has been studied on a glassy carbon electrode using cyclic voltammetric and differential pulse voltammetric methods [30]. An electrochemical sensor for acetylsalicylic detection using both carbon paste electrode and graphite pencil electrode has been described [31].

Dipyrone (DP) is another analgesic, antipyretic, and anti-inflammatory drug. It has been restricted or banned in some countries because of the alleged risks of adverse reactions [32]. Flow injection amperometric and liquid chromatography/mass spectrometry methods have been described for the determination of DP [33–36]. Spectrophotometric methods such as ultraviolet-visible absorption, fluorescence, and chemiluminescence have also been frequently reported for DP determination [37].

The adverse effects of the common drugs either in small or larger doses necessitate the development of newer analytical techniques or modification of existing methods for improved sensitivity. Thus, it is planned to utilize nano-riboflavin-modified electrode for the electroanalysis of the three drugs. This paper reports the stripping voltammetric determination of the above said three common analgesics using nano-RF-modified glassy carbon electrode.

2. Experimental

2.1. Methods and Material. EG&G M 273A Electrochemical Analyzer-Princeton Applied Research Corporation (Princeton, NJ, USA) was employed mainly for carrying out electroanalytical studies. Three analgesic drugs of acetaminophen, acetylsalicylic acid and dipyrone were purchased from SIGMA (St. Louis, Mo, USA) and used as such. Riboflavin was purchased from Merck (KGaA, Darmstadt, Germany). The structure and physical parameters are presented in Table 1. The stock solutions were made up in double distilled TKA-LAB (Niedereiberg, Germany) purified water. For studies in aqueous media, Britton Robinson buffers, 4.0, 7.0, 9.2, and 0.1 mol·dm−3 KOH, KCl, and H2SO4 were used as the medium for the analysis.

2.2. Procedure. Analyte solution kept in electrochemical cell of 15 mL capacity was subjected to purging with high pure nitrogen for 15 minutes under stirring. After purging nitrogen, voltammograms were recorded under blanketing. Great care was taken in the electrode pretreatment to get reproducible results. The glassy carbon electrode was pretreated in two ways: mechanical polishing over a velvet microcloth with an alumina suspension and electrochemical treatment by applying a potential of 1.5 V for 2 seconds.

2.3. Preparation of Nanosize Riboflavin-Modified Glassy Carbon Electrode (RF/GCE). The typical cyclic voltammogram of 0.1 M riboflavin in aqueous 0.1 M solution of KCl medium at the scanning potential 50 mV s−1 is shown in Figure 1. One oxidation peak at −380 mV and one reduction peak at −660 mV were observed in the first cycle. As the number of cycles increased between the potential −800 and 400 mV, the peak current decreased which indicates the absence of further reaction and adsorption of the compound on the working electrode surface. Thickness of the film was controlled coulometrically, and 0.1 μ thick films were used in all cases. The SEM photograph reveals the deposition of nanosize (100 nm) fiber-like structure of RF film on GCE. After each cycle, care was taken to remove the riboflavin film present on the glassy carbon electrode during cleaning of the electrode surface. This was done in a bath containing 1 : 1 HCl/H2O and 1 : 1 H2O2/acetic acid mixture before usual surface treatment procedure which involves mechanical polishing and electrochemical treatments. Nitric acid (6 M) solution was used to clean the cell. The electrode stability of RF film-modified electrode is of prime importance in these studies. The electrode was found to be stable in the medium used for the study. It showed a decrease in peak current after 2 days of...
Table 1: Structure and physical parameters of drugs.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Physical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS No: 103-90-2 Acetaminophen (AAP) (4-Hydroxyacetanilide)</td>
<td><img src="AAP.png" alt="AAP structure" /></td>
<td>Colourless crystalline powder Melting point: 169–170°C Solubility in water, 1.4 g/100 mL at 20°C, Mol. Mass: 151.17 g/mol</td>
</tr>
<tr>
<td>CAS No: 50-78.2 Acetylsalicylic acid (ASA) (2-(acetyloxy)-Benzoic acid;)</td>
<td><img src="ASA.png" alt="ASA structure" /></td>
<td>Colourless crystalline powder Melting point: 136°C with decomposition Solubility in water, 1 g/100 g water at 37°C, Mol. Mass: 180.160 g/mol</td>
</tr>
<tr>
<td>CAS No: 68-89-3 Dipyrone (DP) (1-Phenyl-2,3-Dimethyl-5-Pyrazolone-4-Methylaminomethanesulfonate Sodium)</td>
<td><img src="DP.png" alt="DP structure" /></td>
<td>White crystalline powder Melting point: 172°C Solubility in water: 660.00 g/L with 20°C, Mol. Mass: 311.358 g/mol</td>
</tr>
</tbody>
</table>

Figure 1: Cyclic voltammogram of 0.1 M riboflavin at pH 7.0 on GCE.

Figure 2: Plot of peak potential versus pH.

Figure 3: Plot of peak current versus ν$^{1/2}$.

3. Results and Discussions

3.1. Effect of pH. Cycil voltammograms (CV) of 250 μg mL$^{-1}$ of AAP, ASA and DP were recorded at a sweep rate 100 mV s$^{-1}$ in the five selected pH media (1.0, 4.0, 7.0, 9.2, and 13.0.), and the results were compared to understand the influence of pH on the voltammetric behaviour of the three drugs. AAP and ASA exhibited single oxidation peak in the CV, whereas DP showed three anodic peaks and one cathodic peak. The peak potential with higher peak current was chosen for comparison. The peak potential (Figure 2) and current (Figure 3) were measured and correlated with pH. As the pH increased, the peak potential decreased showing lesser energy requirement for the oxidation in basic medium. Sharpness of peak was very good in pH 1.0. Peak current decreased in neutral condition, and a small increase was found in base medium (Figure 3). For all the three analgesics maximum peak current was noticed at pH 1.0. Hence analytical point of view, pH 1.0 was chosen as the optimum pH.

3.2. Voltammetric Studies of Drugs at pH 1.0. Cyclic voltammetric behaviour of ASA, AAP and DP was studied in the concentration 250 μg mL$^{-1}$ in aqueous pH 1.0 (Figure 4) at scan rate 100 mVs$^{-1}$. The compound ASA and AAP showed only one well-defined anodic peak and DP revealed three anodic and one cathodic peaks. At pH 1.0 AAP and ASA exhibited (Figures 5(a) and 6(a)) one oxidation peak, and DP exhibited (Figure 7(a)) three anodic and one cathodic peaks. The same number of peaks was observed at various sweep rates. Correlation of peak current with sweep rate resulted in a curve line, whereas the plot of ip versus ν$^{1/2}$
3.3. Square Wave Stripping Voltammetric (SWSV) Analysis of Drugs. Cyclic voltammetric results revealed good electroactivity of the drugs on the RF-modified electrode at pH 1.0. Squarewave mode was employed for stripping voltammetric studies and was found to perform well in the determination of all the three analgesics. The accumulation potential was varied between 500 and 750 mV for AAP, 750 and 950 mV for ASA, and 300 and 600 mV for DP. Higher peak response was obtained only at 600, 850, and 550 mV, respectively, for AAP, ASA, and DP. The deposition time was varied between 5 and 60 sec for all drugs and maximum peak current was observed at a deposition time of 10 s for ASA and DP and 30 s for AAP. The accumulation of the three analgesics on the modified electrode surface under the optimum accumulation conditions was understood from the changes in the electrode surface before and after accumulation process. SEM was employed to study the surface morphology of the three accumulated analgesics on the electrode. Figure 8(a) reveals fiber knitting-like structure of GCE and Figure 8(b) shows dense mixed needle like structure of RF-modified electrode surface. The drug AAP showed sponge like structure (Figure 8(c)). ASA adsorbed exhibited knitted fiber-like morphology (Figure 8(d)), and DP exhibited uniformly mixed fiber and sponge like morphology (Figure 8(e)).

The initial scan potential was varied from 0 to 400 mV for AAP, 200 to 700 mV for ASA, and −300 to 300 mV for DP, and optimum value was obtained at 200 mV for AAP and ASA and −300 mV for DP. The stripping peak current increased initially up to 50 mV for ASA and DP and decreased. Hence, this value, 50 mV was considered as optimum. For AAP, increase in square wave amplitude, resulted in a decrease in peak current, and hence, 25 mV was taken as the optimum amplitude.

The dependence of the peak intensity on the frequency was studied between 20 and 100 Hz at a constant value of the step potential of 2 mV. Maximum peak current was observed at 40 Hz. As the frequency increased above 40 Hz, the peak current decreased and the peak was broadened. When the step potential increased to higher value upto 10 mV, a decrease in peak current was noticed. Hence, a frequency of 40 Hz and a step potential of 2 mV were used. The square wave stripping voltammograms obtained under optimum experimental conditions (Table 2) for three analgesics drugs at concentration of 0.1 μg mL$^{-1}$ are given in Figures 5(b), 6(b), and 7(b).

3.4. Analytical Characteristics. The experimental results showed that the peak current increased with the increase in concentration of drugs. A calibration plot was made, and linear dependence of peak current with concentration was observed (Figure 9). The reproducibility of the stripping signal was realized in terms of relative standard deviation for 7 identical measurements carried out at a concentration level of 0.25 μg mL$^{-1}$ (Table 3). The lower limit of detection was found to be 0.016 μg mL$^{-1}$, 0.007 μg mL$^{-1}$ and 0.013 μg mL$^{-1}$ for AAP, ASA, and DP, respectively.
The SWSV method involving riboflavin modified glassy carbon electrode is considered to be the best method for the determination of the analgesics compared to other available methods (Table 4).

Determination of AAP generally suffered from the interference of p-aminophenol as well as ascorbic acid, caffeine, glucose, and urea [23]. Hence, a systematic study of interference due to these compounds was carried out for all the three analgesics. Specificity of the nano-RF-modified electrode to 0.124 μg mL\(^{-1}\) of AAP, ASA, and 0.186 μg mL\(^{-1}\) of DP in the presence of ascorbic acid, caffeine, glucose, p-aminophenol, and urea was checked by recording square wave stripping voltammograms for oxidation of AAP, ASA, and DP after addition of varying concentration of each interferent (0.01–0.1 μg mL\(^{-1}\)). Ascorbic acid, glucose, caffeine, and urea do not affect the peak current of drugs even up to 10-fold-excess of interferent. However, in the case of AAP, oxidation peak due to p-aminophenol started merging

Figure 4: Cyclic voltammogram of (a) ASA, (b) AAP, and (c) DP at pH 1.0 on GCE.
with that of AAP from 0.07 μg mL⁻¹ concentration of the interferent.

3.5. Proposed Method for the Determination of Drugs in Pharmaceutical and Urine Samples. The pharmaceutical samples analyzed were collected from medical shops at Karaikudi, Tamilnadu, India. Various tablets and injection having acetaminophen, acetylsalicylic acid, and dipyrrone were examined for estimation of content of drugs. Stripping voltammograms of the drugs at pH 1.0 were recorded under optimised conditions. The concentration of the analgesics in commercial formulations determined by the proposed method was in good agreement with the reported value of the company (Table 5).

For the measurement of drugs in urine, samples collected after 8 h from the administration of the drug were
Figure 7: (a) Cyclic voltammogram of DP at pH 1.0 on RF/GCE; scan rate 100 mV s\(^{-1}\) (b) SWSV of DP on RF/GCE under optimum conditions.

Table 2: Optimum experimental conditions in SWSV.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ASA Range studied</th>
<th>AAP Range studied</th>
<th>DP Range studied</th>
<th>ASA Optimum value</th>
<th>AAP Optimum value</th>
<th>DP Optimum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1.0 to 13.0</td>
<td>1.0 to 13.0</td>
<td>1.0 to 13.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Accumulation potential (mV)</td>
<td>750 to 950</td>
<td>500 to 750</td>
<td>300 to 600</td>
<td>850</td>
<td>600</td>
<td>550</td>
</tr>
<tr>
<td>Accumulation time (Sec)</td>
<td>5 to 60</td>
<td>5 to 60</td>
<td>5 to 60</td>
<td>10</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Initial scan potential (mV)</td>
<td>200 to 700</td>
<td>0 to 400</td>
<td>–300 to 300</td>
<td>200</td>
<td>200</td>
<td>–300</td>
</tr>
<tr>
<td>Square wave amplitude (mV)</td>
<td>25 to 150</td>
<td>25 to 150</td>
<td>25 to 150</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>20 to 100</td>
<td>20 to 100</td>
<td>20 to 100</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Scan increment (mV)</td>
<td>2 to 20</td>
<td>2 to 20</td>
<td>2 to 20</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Scan rate (mV/sec)</td>
<td>10 to 80</td>
<td>10 to 60</td>
<td>20 to 80</td>
<td>60</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Stirring rate (rpm)</td>
<td>50 to 250</td>
<td>50 to 250</td>
<td>50 to 250</td>
<td>250</td>
<td>250</td>
<td>250</td>
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<tr>
<td>Rest period (Sec)</td>
<td>2 to 10</td>
<td>2 to 10</td>
<td>2 to 10</td>
<td>5</td>
<td>5</td>
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</table>

Table 3: SWSV behaviour of analgesic drugs.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Range studied (μg/mL)</th>
<th>LOD (μg/mL)</th>
<th>% of RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>0.020 to 0.4</td>
<td>0.016</td>
<td>2.3</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>0.020 to 0.4</td>
<td>0.007</td>
<td>2.8</td>
</tr>
<tr>
<td>Dipyrones</td>
<td>0.025 to 0.4</td>
<td>0.013</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 4: Comparison of available methods.

<table>
<thead>
<tr>
<th>Methods</th>
<th>LOD in μg mL(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAP</td>
</tr>
<tr>
<td>Voltammetric determination [28, 29, 37]</td>
<td>0.09–0.93, 38.0</td>
</tr>
<tr>
<td>Flow injection-FT-IR spectrometric [38]</td>
<td>8.0</td>
</tr>
<tr>
<td>HPLC method [39, 40]</td>
<td>31.6</td>
</tr>
<tr>
<td>Spectrophotometric determination [17, 41]</td>
<td>4.0, 0.5–20.0</td>
</tr>
<tr>
<td>Flow injection amperometric determination [35, 42, 43]</td>
<td>20.54</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10.0 mL of the urine sample was mixed with 0.1 M H$_2$SO$_4$ solution, and the pH was adjusted to 1.0. The SWSV was carried out under the optimised experimental conditions. This experiment was repeated for 5 times, and the average weight of drugs in 1.0 mL of urine sample was 0.12 $\mu$g for AAP, 0.23 $\mu$g for ASA, and 0.27 $\mu$g for DP. There was no appreciable interference due to the presence of other compounds present in the urine. There was no degradation of the drug in solution during the experiments. The method described is simple and suitable for the determination of drugs. Repetition rate was high. Hence, the proposed method can be used as a better alternative to spectrophotometric or chromatographic methods.

### 4. Conclusion

Electrooxidation of three analgesics AAP, ASA, and DP showed irreversible two electron transfer oxidation on nano-RF-modified glassy carbon electrode in the pH range 1.0 to 13.0. The oxidation of drugs was controlled by diffusion. The study on the influence of pH revealed pH 1.0 as the best pH for the development of analytical procedure. Detailed square wave stripping voltammetric studies were carried out at pH 1.0, and optimum accumulation and stripping conditions were arrived at. The concentration was varied under optimum experimental conditions, and calibration was made. From the SEM photographs, accumulation of drugs was understood. Lower limit of detection for all
Table 5: Amount of drugs in tablets determined by SWSV in tablets.

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Company name</th>
<th>Tablets (mg)</th>
<th>Experimental value (mg)</th>
<th>% of RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acteaminophen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calpol</td>
<td>Glaxosmithkline</td>
<td>500</td>
<td>492</td>
<td>2.5</td>
</tr>
<tr>
<td>Crocin</td>
<td>Glaxosmithkline</td>
<td>500</td>
<td>495</td>
<td>3.1</td>
</tr>
<tr>
<td>Colimex tabs</td>
<td>Wallace</td>
<td>500</td>
<td>494</td>
<td>2.3</td>
</tr>
<tr>
<td>Doliprane</td>
<td>Nicholas piramal</td>
<td>500</td>
<td>498</td>
<td>2.7</td>
</tr>
<tr>
<td>Cyclopam</td>
<td>Indoco</td>
<td>500</td>
<td>498</td>
<td>2.2</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Disprin</td>
<td>Reckitts</td>
<td>350</td>
<td>347</td>
<td>3.1</td>
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<tr>
<td>Myncopyrin</td>
<td>Nicholas piramal</td>
<td>350</td>
<td>346</td>
<td>2.8</td>
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<tr>
<td>Asa50</td>
<td>German remedies</td>
<td>50</td>
<td>45</td>
<td>2.2</td>
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<tr>
<td>Colsprin100</td>
<td>Reckitts</td>
<td>100</td>
<td>97</td>
<td>3.3</td>
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<td>Ecosprin</td>
<td>Sidmak</td>
<td>150</td>
<td>148</td>
<td>2.5</td>
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<tr>
<td>Dipyroprone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novalgin</td>
<td>Aventis</td>
<td>500</td>
<td>498</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Figure 9: Calibration plot of peak current versus concentration.

the three analgesics are reported. This method can very well be used for the determination of three drugs in real samples also. This technique is simple and easy to carry out. LOD values from this study for all the three analgesics are compared with the reported values. On comparison, the proposed method is found to be best method for the determination of AAP, ASA, and DP.

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