Spectrophotometric determination of tramadol-HCl based on the its reaction with 1-naphthylamine and sodium nitrite in the presence of SDS

ABSTRACT

Tramadol hydrochloride is a centrally acting analgesic, used for treating moderate to severe pain. Tramadol hydrochloride possesses agonist actions at the μ-opioid receptor and effects reuptake at the noradrenergic and serotonergic systems. Chemically it is \[\text{[2- (dimethylaminomethyl)-1-(3-methoxyphenyl) cyclohexanol]}\]. Literature survey reveals that, several spectrophotometric methods used for drug determinations. In this work, a spectrophotometric method is proposed for determination of tramadol-HCl. Tramadol-HCl reacts with 1-naphthylamine and sodium nitrite, after heating for 180s at 80°C to give an orange red color solution having maximum absorbance at 460 nm. The effect of anionic surfactant sodium dodecyl sulfate (SDS) on the reaction was examined. The effect of different parameters were studied and optimized. In the optimum conditions, the linear range was obtained as 10 to 300 µg/ml, while the relative standard deviation is 1.17% and the reaction is selective for tramadol with 1 ng/ml as visual limit of identification. The results obtained showed good recoveries. The proposed procedures were applied successfully to analysis of tramadol in its pharmaceutical preparations and results were favorably comparable with the official method.

Keywords: Tramadol, spectrophotometric analysis, real sample analysis, SDS, 1-naphthylamine.

INTRODUCTION

Study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample (Chemistry 111 Lab, 2005).

Tramadol (marketed as Ultram and generics) is an opioid pain medication which is used to treat moderate to moderately severe pain (British Pharmacopoeia, 2003). When taken as an immediate-release oral formulation, the onset of pain relief usually occurs within about an hour (Abdellatef et al., 2006). It has two different mechanisms. First, it binds to the μ-opioid receptor. Second, it inhibits the reuptake of serotonin and norepinephrine (Pedersen et al., 2003; Gan and Ismail, 2001). Serious side effects may include seizures, increased risk of serotonin syndrome, decreased alertness and drug addiction. Common side effects include: constipation, itchiness and nausea, among others. A change in dosage may be recommended in those with kidney or liver problems.

Tramadol is marketed as a racemic mixture of both R- and S-stereoisomers (British Pharmacopoeia, 2003); this is because the two isomers complement each other’s analgesic activity (British Pharmacopoeia, 2003). It is often combined with paracetamol (acetaminophen) as this is known to improve the efficacy of tramadol in relieving pain (British Pharmacopoeia, 2003). Tramadol is metabolized to O-desmethylltramadol, which is a more potent opioid
Tramadol is recommended for the management of pain in fibromyalgia by the European League against Rheumatism (Cao et al., 2002). Its analgesic effects take about one hour to come into effect and 2 to 4 h to peak after oral administration with an immediate-release formulation (Valli et al., 2001). On a dose-by-dose basis tramadol has about one-tenth the potency of morphine and is approximately equally potent when compared to pethidine and codeine (Garrido et al., 2003). For pain moderate in severity its effectiveness is equivalent to that of morphine; for severe pain it is less effective than morphine (Valli et al., 2001). The most common adverse effects of tramadol include nausea, dizziness, dry mouth, indigestion, abdominal pain, vertigo, vomiting, constipation, drowsiness and headache (Wu et al., 2005; Abu-Shawish et al., 2010).

Compared to other opioids, respiratory depression and constipation are considered less of a problem with tramadol (Abu-Shawish et al., 2010). Different methods for the analysis of the selected drug have been reviewed. The BP (Brayfield, 2014) specifies non-aqueous titration technique detecting the end point potentiometrically for its determination. The literature reveals several methods for the determination of the mentioned drug in biological fluids and pharmaceutical preparations. Among these methods are spectrophotometry (Ultram, 2013; Product Information Tramadol, 2011; Katz, 1996), HPLC (Leppert, 2009; Raffa et al., 2012) GC (Rossi, 2013), LC-MS/MS (Grond and Sablotzki, 2004), capillary electrophoresis (Carville et al., 2008), voltammetry (Lee et al., 1993) and potentiometry (Micó et al., 2006; Bloor et al., 2012; Tramadol, 2009; Langley et al., 2010; Keating, 2006; Collett, 2001).

An inspection of the previous methods for the determination of the cited drug revealed that only a few spectrophotometric ones have been reported. Although atomic absorption spectrometry (AAS) is a rapid technique and has a low detection limit, it has not been yet applied to the determination of tramadol, the same case being with the conductometric procedures which proved to be simple, sensitive, reliable, very convenient and a simple procedure. During a systematic study of drugs of abuse (Aman et al., 1993, 1992; Aman, 1994, 1995; Koupparis et al., 1985) it was found that tramadol reacts with 1-naphthylamine and sodium nitrite to give an orange color having maximum absorbance at 460 nm. The reaction obeys Beer’s law and has 1 ng/ml-1 as visual limit of quantitation. This colour reaction is not reported in the literature. The present method is simple, accurate, precise and sensitive. Percentage of tolerable limits of other drugs not interfering is also studied.

In this work, a spectrophotometric method is proposed for the determination of tramadol-HCl. Tramadol-HCl reacts with 1-naphthylamine and sodium nitrite after heating for 180 s at 80°C to give an orange red color having maximum absorbance at 460 nm. The effect of anionic surfactant sodium dodecyl sulfate (SDS) on the reaction was studied. The effect of different parameters were studied and optimized. In the optimum conditions, the linear range was obtained as 10 to 300 mg/L. Finally, the proposed method was applied for determination of tramadol-HCl in different real samples.

MATERIALS AND METHODS

Apparatus

UV–Vis absorbance spectra were collected using a Lambda2 Perkin-Elmer spectrophotometer and 1-cm quartz cells. The recorded spectra were digitized with one data point per nanometer. Measurements of pH values were made with Met Rohm 780 pH-meter using a combined glass electrode.

Reagents

All materials and reagents used were of analytical grade; solvents were of spectroscopic grade and bidistilled water was used. Tramadol hydrochloride pure drug and tramadol capsules (labelled to contain 50 mg tramadol hydrochloride per capsule) were obtained from HelalAhmar Drugstore, Urmia, Iran. All chemicals used in the experiments were of analytical grade and were used without further purification. 1-naphthylamine, sodium nitrite, sodim dodecyl sulfate (SDS), trisodium citrateno-hydrate and methanol were obtained from Merck (Darmstadt, 91 Germany). Acetate buffer, pH 4.8 was prepared by dissolving 10 g of anhydrous sodium acetate in 300 ml water, adjusting pH to 4.8 with glacial acetic acid and diluting to 1000 ml with deionized water (DDW). If necessary, pH was readjusted to the value of 4.8 with glacial acetic acid or anhydrous sodium acetate as required before use.

Standard drug solutions

Aqueous solution of 0.1 and 4 mg/ml tramadol hydrochloride was prepared by dissolving 10 and 400 mg of the pure drug in 100 ml deionized water, respectively. Working solutions of lower concentrations were prepared by appropriate dilution of the standard solutions.

Spectrophotometric procedures

At first, different amounts of SDS 2% were established; sodium nitrite 4% and 1-naphtilamin 1% in one at the time
a test tube and this process was done to reach optimum amount of mode Add-in these solution after aliquots containing 1 ml 10⁻² mol/L of tramadol drug solution was added to the mixture. Also, the solution reached a volume of 10 ml and stood for another 9 min in water bath in fixed 80°C temperature. At last, this solution was added into spectrophotometer cell to take spectra. The absorbance of the solution at 460 nm (Figure 1) was then measured against a reagent blank prepared according to the same treatment.

Serum sample treatment

Human blood samples were obtained from healthy volunteer and patient ones that consumed the tramadol into prepare serum samples in Imam Khomeini hospital (Urmia City, Iran). They were drawn into the test tube, centrifuged at 2500 rpm for 10 min and then allowed to stand at 4°C until the phase separation was done. The serum samples were kept in a freezer (-30°C) until analysis of a 400 µl of each serum sample was transferred into a 25 ml volumetric flask and diluted to the mark with deionized water.

Tablet sample preparation

For purpose of pre-treatment, the average content of tramadol tablets was calculated from the contents of 5 tablets each. The contents were then finely ground and a portion of the powder was weighed accurately, transferred into a 50 ml flask and diluted to scale with water. The mixture was sonicated for 8 min for appropriate dissolution and then filtered. An accurate volume of the filtrate was further diluted with DDW so that the concentration of tramadol in the final solution could meet the linear range of the working-curve.

RESULTS AND DISCUSSION

Effect of pH on determination procedure

One of the important parameters in this process is the pH. It is very obvious that the structure of the drugs and carrier particles depends on the pH. It is clear in the pH chart that the best condition of the determination occurs in the pH of about 4.8, and as mentioned the absorption spectrum of mixture with drugs injected in that is by placing several layers of material near the surface of mixture, while absorbance spectra slowly shifts and depress and the complexation of drugs with mixture occur better (Curticapean et al., 2008; Valli et al., 2001; Patel et al., 2009; Cao et al., 2002). However, the drugs structure can also be affected by the changes in pH. Determination of drugs becomes easier and the physiological and consumption solutions and tablets pH of these drugs is between 4 to 7 (Abdellitef, 2002). For these reasons, pH of mixture mixed was investigated between 2 to 12 and optimum pH of 4.8 was chosen (Figure 2).

In order to obtain the presence or absence of SDS and how its affects the result; we take spectra with and without SDS; this spectra proved that using SDS is not very effective in the determination process (Figure 3).

Effect of concentration of SDS

The SDS will disperse as very fine droplets when rapidly injected in the aqueous sample. The main parameter for selecting this solvent volume is its miscibility with water. Different volumes of SDS (0.1 to 1.3 ml) were tested to obtain the best results. Figure 4 shows that the absorbance intensity increased with increasing volume of SDS to 0.8 ml. However, the mixture can support tramadol better with increasing of SDS volume. It is clear that by increasing the volume of SDS, the solubility of the complex in water increases, therefore, the absorbance intensity also increase.

Effect of concentration of 1-Naphtile Amin

The influence of the 1-Naphtile Amin volume on the determination efficiency was evaluated in the volume range of 0.4 to 2 ml (1.0%). The results are illustrated in Figure 5. As it is shown, the absorbance intensity of the mixture was increased with increasing 1-Naphtile Amin volume up to
Effect of concentration of sodium nitrite

In most traditional processes salt was often added into the sample solution to improve the efficiency. Generally, addition of sodium nitrite increases the solubility of analytes in the aqueous sample and enhances one another. For investigating the influence of sodium nitrite volume on performance of our method various experiments were performed by adding different volumes of sodium nitrite (0.3 to 1.4 M) (Figure 6). Other experimental conditions were kept constant. The results showed that the best result was slightly increased by the addition of sodium nitrite volume of 1.4 ml. It is obvious that with increasing 1-Naphtile Amin volume, the complexion was increased which resulted in further increase in volume of the reagent and showed no significant change in efficiency. Thus, a 1-Naphtile Amin volume of 1.4 ml was chosen for subsequent experiments.

Effect of concentration of sodium nitrite
Effect of concentration of sodium nitrite on determination of tramadol (mixture contain 0.8 ml SDS 2%, 1.4 ml 1-naphthile amine 1.0%, 0.3 to 1.4 ml sodium nitrite 4%, pH 4.8, temperature 80°C, time 9 min and 1 ml tramadol from solution 10^{-7} mol/L).

Effect of temperature

In all processes that aqueous solution was used, solubility appeared better with increasing in temperature, but further increase of temperature was not very useful. Because water can start boiling and determination process gets very difficult, temperature from 0 to 100°C were examined and 80°C selected as the best temperature for the process (Figure 7).

Effect of time

The time of mixing of mixture is one of the most important factors in this procedure, especially in liquid-liquid interactions. In this process, time is defined as the time between injection of a mixture in test tube to taking from water bath, and the time of start of centrifugation. The time was examined in the range of 3 to 15 min with keeping other experimental conditions constant. As shown in Figure 8 the time attained was within 9 min.

Method of validation

For these purpose, under the optimum experimental conditions, a typical calibration curve was obtained for the determination of tramadol by plotting ∆A signal vs. tramadol concentrations. The calibration curve was linear in the range of 10 to 300 µg/ml with y=0.0725x+521.92, R^2=0.9959 and the detection limit is 1.0 ng/ml (n=6) for tramadol. Table 1 shows a comparison between the results obtained by the present method with those obtained by some other methods reported for the determination of tramadol. As compared to Table 1, the present method has a good detection limit and the linear range compared with Liquid Chromatography (LC), High performance liquid chromatography (HPLC), electrochemical and Gas chromatography (GC) methods (Mohammad-Reza et al., 2006; Cecatto et al., 1996; Saciloto et al., 2013; Hisham, 2002; Cecatto et al., 2000; Lintz and Uragg, 1985). It should be highlighted that the major advantages of this method is using of very simple method for determination of this drug.

Interference study

The influences of foreign coexisting substances such as morphine, ascorbic acid, codeine, Ibuprofen, saccharides, amino acids and ions were tested. As listed in Table 2, most of the examined coexisting substances had remarkable interference on the assay. From the results, the interference of K⁺, Na⁺, NO₃⁻, Zn²⁺, Ni ²⁺, Al ³⁺, Fe ²⁺, Mn²⁺, tryptophan, tyrosine, glucose, sucrose, fructose and lactose were very weak. Among the tested substances Cu²⁺, Cd²⁺, SO₄²⁻, I⁻, Cl⁻,
Table 1: Characteristic performance data obtained by spectrophotometric method and other techniques for determination of tramadol.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Linear range (µg/ml)</th>
<th>LOD (µg/ml)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>0.001-0.5</td>
<td>0.005</td>
<td>(Mohammad-Reza et al., 2006)</td>
</tr>
<tr>
<td>SPE-LC</td>
<td>0.0025-0.15</td>
<td>0.05</td>
<td>(Ceccato et al., 1996)</td>
</tr>
<tr>
<td>Voltammetric method</td>
<td>50-250</td>
<td>0.1</td>
<td>(Saciloto et al., 2013)</td>
</tr>
<tr>
<td>Kinetic spectrophotometric method</td>
<td>1-250</td>
<td>0.012</td>
<td>(Hisham, 2002)</td>
</tr>
<tr>
<td>LC-TMS</td>
<td>0.08-50</td>
<td>0.0014</td>
<td>(Ceccato et al., 2000)</td>
</tr>
<tr>
<td>GC-MS</td>
<td>25-200</td>
<td>1.0</td>
<td>(Lintz and Uragg, 1985)</td>
</tr>
<tr>
<td>This work</td>
<td>10-300</td>
<td>0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

*a*high performance liquid chromatography; *b*Solid-phase extraction-Liquid chromatography; *c*Applying Liquid Chromatography-Tandem Mass Spectrometry and *d*Gas chromatography-mass spectrometry.

Table 2: Tests for interference substance on Tramadol determination (mixture contain 0.8 ml SDS 2%, 1.4 ml 1-naphthylamine 1.0%, 1.3 ml sodium nitrite 4%, pH 4.8, temperature 80°C, time 9 min and 1 ml tramadol from solution 10-7 mol/L).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (µg/ml)</th>
<th>Change in intensity of absorbance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg²⁺, Fe³⁺, Ca²⁺</td>
<td>120.0</td>
<td>24</td>
</tr>
<tr>
<td>K⁺, Na⁺, NO₃⁻</td>
<td>100.0</td>
<td>4</td>
</tr>
<tr>
<td>I⁻, Cl⁻</td>
<td>160.0</td>
<td>35</td>
</tr>
<tr>
<td>Zn²⁺, Ni²⁺, Al³⁺, Fe²⁺, Mn²⁺</td>
<td>162.0</td>
<td>6</td>
</tr>
<tr>
<td>Cu²⁺, Cd²⁺, SO₄²⁻</td>
<td>159.0</td>
<td>15</td>
</tr>
<tr>
<td>Morphine, codeine, Ibuprofen</td>
<td>600.0</td>
<td>40</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>176.0</td>
<td>30</td>
</tr>
<tr>
<td>Cysteine</td>
<td>120.0</td>
<td>16</td>
</tr>
<tr>
<td>Tryptophan, tyrosine</td>
<td>204.0</td>
<td>5</td>
</tr>
<tr>
<td>Glucose, sucrose, fructose, lactose</td>
<td>180.0</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3: Analysis of diluted blood serum sample and tablet for tramadol (mixture contain 0.8 ml SDS 2%, 1.4 ml 1-naphthylamine 1.0%, 1.3 ml sodium nitrite 4%, pH 4.8, temperature 80°C, time 9 min and 1 ml tramadol from solution 10-7 mol/L).

<table>
<thead>
<tr>
<th>Added (µg/ml)</th>
<th>Expected (µg/ml)</th>
<th>Found (µg/ml)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>0.29</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>10.00</td>
<td>10.29</td>
<td>10.40</td>
<td>2.51</td>
<td>101.1</td>
</tr>
<tr>
<td>20.00</td>
<td>20.29</td>
<td>20.00</td>
<td>1.92</td>
<td>98.55</td>
</tr>
<tr>
<td>30.00</td>
<td>30.29</td>
<td>30.58</td>
<td>1.53</td>
<td>100.96</td>
</tr>
<tr>
<td>40.00</td>
<td>40.29</td>
<td>40.18</td>
<td>2.12</td>
<td>99.72</td>
</tr>
<tr>
<td>b) tablet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50.00</td>
<td>50.51</td>
<td>1.91</td>
<td>101.0</td>
</tr>
<tr>
<td>10.00</td>
<td>60.00</td>
<td>61.15</td>
<td>2.35</td>
<td>111.5</td>
</tr>
<tr>
<td>20.00</td>
<td>70.00</td>
<td>71.05</td>
<td>2.14</td>
<td>105.25</td>
</tr>
<tr>
<td>30.00</td>
<td>80.00</td>
<td>78.89</td>
<td>2.41</td>
<td>96.30</td>
</tr>
</tbody>
</table>

Mg²⁺, Fe³⁺ and Ca²⁺ and cysteine can be allowed with relatively higher concentrations, while morphine, codeine, Ibuprofen and ascorbic acid can only be allowed with relatively low concentrations. The allowed concentrations of these interfering substances however, were still rather higher than that of tramadol which indicated that this method had a high selectivity.

**Real sample analysis**

In order to test the applicability of the proposed method, it was applied to determine tramadol in serum and tablet samples. Table 3a and b shows that tramadol detected in the original serum was about 0.29 and 50.00 µg/ml respectively. The average recovery of tramadol was in the
range of 98.55 to 101.10 and 96.30 to 111.5%, respectively. The RSD of six replicate determinations was not higher than 2.51%. The aforementioned results demonstrated the potential applicability of these methods for the detection of tramadol in serum sample. The results for the assay of tramadol are given in Table 3. The relative standard deviation was less than 2.4% for tablet analysis and the recoveries were illustrated and the good performance of the proposed method for determination of tramadol in serum and tablet samples.

Conclusion

The data presented revealed that the proposed methods introduced new techniques for the determination of tramadol. The proposed methods are simple, accurate and sensitive with good precision and accuracy. With these methods, one can do the analysis in a short time at low cost without losing accuracy. The proposed methods can be used as alternative methods to reported ones for the routine determination of tramadol in the pure form and in pharmaceutical formulations.

REFERENCES


