SPECTROFLUORIMETRIC DETERMINATION OF TRAMADOL IN FORMULATION AND BIOLOGICAL FLUIDS

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ABSTRACT

A simple and reliable spectrofluorimetric method has been developed for the determination of tramadol HCl in the pharmaceutical preparation and biological fluids. The fluorescence of the product was found to have excitation at $\lambda_{\text{max}}$ 272 nm and emission $\lambda_{\text{emi}}$ at 298 nm. The method shows high sensitivity with linearity range from 0.07 to 0.4 $\mu$g/mL. The lower limit of detection (LOD) was found to be $1.127 \times 10^{-3}$ $\mu$g/mL, $4.202 \times 10^{-3}$ $\mu$g/mL and limit of quantification (LOQ) was found to be $3.415 \times 10^{-3}$ $\mu$g/mL, $1.273 \times 10^{-2}$ $\mu$g/mL in formulation and biological fluids, respectively. The different experimental parameters affecting the fluorescence intensity were carefully studied and optimized. The proposed method was applied successfully for determination of tramadol in the pharmaceutical preparation and biological fluids. The percentage recovery was found to be 99.477 ± 0.73 and 102.48 ± 1.433 for formulation and biological fluids, respectively.

Key words: Tramadol, Fluorimetry, Biological fluid.

INTRODUCTION

Tramadol hydrochloride is (±) cis – 2 – [(dimethylamino) methyl] – 1 – (3 – methoxy phenyl) – cyclohexanol hydrochloride. It is an atypical opioid, which is a centrally acting analgesic, used for treating moderate to severe pain and most types of neuralgia, including trigeminal neuralgia. It is a synthetic agent as a phenyl piperidine analogue of codeine1. It is suggested that tramadol could be effective for alleviating symptoms of depression and anxiety because of its action on GABA aergic, noradrenergic and specifically serotonergic systems2. The (+) – enantiomer is approximately four times more potent in terms of ~lopioid receptor affinity and 5-HT reuptake, whereas the (-)-

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enantiomer is responsible for noradrenaline reuptake effects. (+) – Tramadol exhibiting ten – fold higher analgesic activity than (-) – enantiomer.

Its therapeutic plasma concentration is in the range of 100-300 ng/mL, rapidly and almost completely absorbed after oral administration with an absolute bioavailability of only 65-75% due to first pass metabolism. This analgesic is rapidly and extensively metabolized in the liver. It is well absorbed in oral administration. Co-administration of tramadol with food has no clinically significant effect on rate or extent of absorption, less protein bound (20%), undergoes hepatic demethylation and glucuronidation. It also tries to establish relation between chemical structure and biological activity and to link the later to the physical properties of the drug molecules. Half life of the drug is 5 – 7 h and it is excreted by renal.

It is administered at dosages of 50-400 mg daily, with up to 600 mg daily. When given by the IV/IM route, it has a mean absolute bioavailability of approximately 75% of following administration of a single 100 mg oral dose tramadol tablets. The adverse effect of tramadol includes drowsiness, nausea, vomiting and sweating. Studies have already been reported on this drug analysis by UV – Visible Spectrophotometry, GC-MS, capillary gas chromatography – mass spectrometry, capillary zone electrophoresis – electrospray ionization mass spectrometry, HPLC method using the UV detector in plasma by diode array detector and UV-Visible detector, ion trap mass spectrometry and stereospecific HPLC method, tandem mass spectrometry, LC – MS/MS, electrochemical were reported. This paper describes a simple and reliable method for assaying tramadol by spectrofluorimeter, which has been used to analyze the formulation and plasma concentrations of tramadol in a patient.

**EXPERIMENTAL**

**Chemicals and reagents**

Tramadol sample was supplied by M/s Unichem Ltd, India as gift sample and used as such. Methanol used was spectro grade from S.D fine chemicals Ltd, India. Ethyl acetate used was Analytical Reagent grade from Merck Ltd., India. Water used was HPLC grade generated from Milli-RO 10 plus Milli-Q purification system. (Milli Q Academic from Millipore (India) Pvt. Ltd. All other chemicals used were of analytical reagent grade supplied by M/S Fisher Inorganics and Aromatics Ltd, India.
Standard solutions

Stock solution

Tramadol (100 mg) was accurately weighed and dissolved in 100 mL of methanol and used as stock solution. Further dilutions were made with methanol to get required concentration. For linearity study, serial dilutions were made for tramadol in the range of 0.07 to 0.4 mcg/mL concentrations and solutions were prepared by diluting the stock solution with methanol. For each concentration, six replicates were made by individual weighing. The solutions were investigated in spectrofluorimeter by keeping the excitation as 272 nm and emission as 298 nm.

For formulation

The average weight of the tablets were determined by weighing 20 tablets and there were powdered. Tablet powder equivalent to 50 mg of tramadol was weighed and transferred to a 100 mL volumetric flask. About 60 mL of methanol was added and it was sonicated for 15 minutes for complete dissolution of drugs and then made up to the volume with methanol and filtered through filter paper. Dilutions were made with methanol to attain a concentration of 0.3 µg/mL and spectrum was recorded. Six replicates of analysis were carried out with sample weighed individually. The average weight of the tablet was found to be 0.31118 g.

For biological fluids

About 100 mg of tramadol powder was accurately weighed and taken into a 100 mL volumetric flask, dissolved in methanol and made up to the volume with methanol. A series of dilution was made with methanol to get the concentrations of 3.5, 7.0, 10.5, 14.0, 17.5 and 21.0 µg/mL. From each concentration, 100 µL of drug solution was transferred into a centrifuge tubes to which 900 µL of plasma was added. Then it was vortexed for 1 min for effective mixing of drug solution and plasma, so that the final concentration will be 0.07 to 0.4µg/mL. To the above mixture, 5 mL of ethyl acetate was added and vortexed for 15 minutes on a rocking platform at moderate speed, followed by centrifugation at 1500 rpm for 10 min. Four mL of the supernatant fluid (organic solvent) was withdrawn and transferred to a 5 mL screw thread tapered disposable borosilicate centrifuge tube and evaporated to dryness under reduced pressure at room temperature in a vacuum centrifuge for 45 min. The residue was reconstituted in 2 mL of methanol, vortexed for 10 min and filtered through membrane filter (0.5 µ). The filtrate was used for analysis. For each concentration, six replicates were made by individual weighing. The spectrum was recorded by keeping 272 as λ_{max} for tramadol and emission was scanned between 280 and
450 nm. Fluorescence maxima were observed at 328 as $\lambda_{emi}$ for tramadol and readings were noted. The calibration graphs were constructed taking mean fluorescence value at $\lambda_{emi}$ on Y axis and concentration on X axis. The regression coefficient and intercept were calculated. The spectra of the solution were used for further linearity studies.

**Calibration curves**

Calibration standards of tramadol, covering the range 0.07–0.4 mcg/mL, were prepared by spiking drug free plasma with the suitably diluted tramadol, subjected to the extraction procedure and spectrum was taken as described above. The calibration curves were obtained by plotting the intensity of fluorescence against concentration of tramadol in spiked plasma. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

**Method validation**

Method validation was performed in terms of specificity and selectivity, precision and accuracy, linearity and stability.

**Specificity and selectivity**

The interference from endogenous compounds was investigated by the analysis of six different blank matrices.

**Precision and accuracy**

Method validation regarding reproducibility was achieved by replicate injections of extracted standard solutions at low, medium and high concentration levels, where intensity of fluorescence was measured in comparison to the intensity of fluorescence of the standard. Intermediate precision study (day–to–day reproducibility) was conducted during routine operation of the system over a period of six consecutive days. Statistical evaluation revealed relative standard deviations at different values of six replicates. Within – day repeatability was studied by six replicates at three concentration levels.

**Stability**

Problems of stability are usually encountered with these compounds, mainly affecting analyte in plasma at room temperature. From blood sampling to analysis, storage in the freezer eliminates decomposition. The stability of tramadol was verified by storing sample solutions refrigerated for 6 months. Concentrations were measured once a week. For formulation, the sample solutions were prepared and analyzed for 4 h in the interval of
30 min. and it was found that the differences are within the limit.

**RESULTS AND DISCUSSION**

**Calibration curves**

Calibration standards for tramadol, covering the range 0.07 – 0.4 µg/mL, were prepared by serial dilutions with methanol for pure drug and in 1 mL of drug free plasma was subjected to the extraction procedure indicated above for biological fluids. The calibration curve was obtained by plotting the intensity of fluorescence of the tramadol versus analyte concentration.

**Table 1. Analysis of tablet formulation**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Biological fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug content (mg/tab)</td>
<td>% Label claim</td>
</tr>
<tr>
<td>51.58101</td>
<td>103.162</td>
</tr>
<tr>
<td>51.27133</td>
<td>102.5427</td>
</tr>
<tr>
<td>50.46452</td>
<td>100.929</td>
</tr>
<tr>
<td>50.65189</td>
<td>101.3038</td>
</tr>
<tr>
<td>49.54628</td>
<td>99.09256</td>
</tr>
<tr>
<td>49.37189</td>
<td>98.74378</td>
</tr>
<tr>
<td>50.48115</td>
<td>100.9623</td>
</tr>
<tr>
<td>0.890836</td>
<td>1.781672</td>
</tr>
</tbody>
</table>

**Table 2. Accuracy of tramadol**

<table>
<thead>
<tr>
<th>Level added (%)</th>
<th>Recovery (%)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>98.56952</td>
<td>1.094108</td>
</tr>
<tr>
<td>50%</td>
<td>100.2104</td>
<td>0.331588</td>
</tr>
<tr>
<td>75%</td>
<td>99.65493</td>
<td>0.766508</td>
</tr>
</tbody>
</table>

*Mean of three determinations
Fig. 1: Regression analysis of the calibration curve for tramadol showed a linear relationship between the intensity of fluorescence and the concentration, with correlation coefficients higher than 0.9944 in all the curves assayed.

Fig. 2: Regression analysis of the calibration curve for tramadol in plasma showed a linear relationship between the intensity of fluorescence and the concentration, with correlation coefficients higher than 0.9994 in all the curves assayed.
The slope and intercept of the calibration line were determined by linear regression using the least squares method. In Fig. 1 and 2, regression analysis of the calibration curve showed a linear relationship between the intensity of fluorescence of tramadol and the concentration, with correlation coefficients higher than 0.9944 and 0.9994 in all the curves assayed in pure form and in biological fluid, respectively. The precision and accuracy of the assay are presented in Tables 1 and 2.

**Selectivity and specificity**

The drug tramadol in the formulation and the plasma was well identified under this condition. No interferences was observed in six different blank plasma samples of tramadol. Fig. 1 shows the regression analysis of the calibration curve for tramadol in pure form, which given showed a linear relationship between the intensity of fluorescence and the concentration, with correlation coefficients higher than 0.9944 in all the curves assayed. Fig. 2 shows the regression analysis of the calibration curve for tramadol in plasma and it showed a linear relationship between the intensity of fluorescence and the concentration, with correlation coefficients higher than 0.9994 in all the curves assayed. Fig. 3 shows the absorption spectrum of tramadol and Fig. 4 shows fluorescence spectrum of tramadol at 272 nm as excitation.

![Absorption spectrum of tramadol](image-url)
Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD determined as the amount of drug was found to be $1.127 \times 10^{-3} \, \mu g/mL$ in formulation and $4.202 \times 10^{-3} \, \mu g/mL$ in biological fluid. The LOQ, determined as the lowest concentration, was found to be $3.415 \times 10^{-3} \, \mu g/mL$, $1.273 \times 10^{-2} \, \mu g/mL$ in formulation and in biological fluid, respectively.

Robustness

The method has been used by two different analysts, with between person variability, within the range of inter-assay variabilities observed for the same analyst. For the lack of resources, the method could not be repeated in a different laboratory or using different equipment. Accuracy was estimated as the deviation to the observed mean concentration from actual concentration and found to be less than 2% for all the concentrations.

CONCLUSIONS

A spectrofluorimetric method for quantifying tramadol in formulation and plasma
samples has been developed and validated in human plasma. The assay is selective, precise, accurate and linear over the concentration range studied. Using 1 mL of plasma, concentrations of tramadol is as low as $3.415 \times 10^{-3} \, \mu g/mL$ in formulation and $1.273 \times 10^{-2} \, \mu g/mL$ in plasma respectively could be precisely quantified and LOD was approximately $1.127 \times 10^{-3} \, \mu g/mL$ in formulation and $4.202 \times 10^{-3}$ $\mu g/mL$ in plasma respectively. The method is simple and suitable for the determination of tramadol in formulation and plasma in pharmacokinetic studies.

**ACKNOWLEDGEMENT**

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**REFERENCES**

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