A simple colorimetric method for estimation of tramadol hydrochloride in pure and tablet dosage forms

Scaria P. Thomas, Hari K. N. Sankar

Abstract:

Objective: The objective of this study was to develop and validate a simple method for estimation of tramadol hydrochloride (TH) in pure and pharmaceutical dosage forms using a colorimeter.

Materials and Methods: TH on reaction with Eriochrome Black T in the presence of acetate buffer at pH 3.5 forms a colored complex. This complex was extracted with a fixed volume of chloroform. The optical density of this colored complex was measured against reagent blank using a colorimeter at 520 nm.

Results: Beer’s law was obeyed with a good correlation coefficient (0.999) in the concentration range of 2.5 µg/ml to 10 µg/ml. Drug content estimation and recovery studies carried out on commercial tablet dosage forms demonstrated the accuracy of the method and that excipients do not cause interference. Precision and robustness were measured and found to be acceptable (% relative standard deviation <2%).

Conclusion: The proposed method can be used for the rapid determination of TH content in tablets at a health-care provider level using already available staff and equipment.

Key words: Colorimetry, Eriochrome Black T, tramadol

Tramadol hydrochloride (TH) is the salt of tramadol which is a centrally acting analgesic that acts as a weak agonist at the μ-opioid receptor. A part of its analgesic effect is produced by inhibition of uptake of norepinephrine and serotonin. It is used in the management of moderate to moderately severe pain. Its respiratory depressant effect is less than that of morphine. The oral bioavailability of tramadol after a single oral dose is 68%. Tramadol undergoes extensive hepatic metabolism by a number of pathways, including CYP2D6 and CYP3A4, as well as by conjugation with subsequent renal excretion. The primary O-demethylated metabolite is two to four times more potent than the parent drug and may account for part of the analgesic effect. The elimination half-life is 6 h for tramadol and 7.5 h for its active metabolite. The important adverse effects include seizures and risk of serotonin syndrome. Tramadol-induced analgesia is not entirely reversible by naloxone, but tramadol-induced respiratory depression is reversed by naloxone. The chemical name for TH is 2-(dimethylaminomethyl)-1-(3-methoxyphenyl) cyclohexanol hydrochloride. Its molecular formula is C_{16}H_{25}NO_2. The structural formula is given in Figure 1.

TH is a white, crystalline, bitter, and odorless powder with a molecular weight of 299.8. It is readily soluble in water and ethanol and has a pKa of 9.41. There are more than 35 commercial formulations of TH and are available as tablets containing 50 mg or 100 mg, capsules containing 50 mg or 100 mg, injections containing 25 mg or 50 mg per ml and 100 mg suppository.

Literature review reveals that several analytical methods have been reported for the estimation of TH alone or in combination with other drugs which include spectrophotometry, spectrofluorimetry, high-performance thin-layer chromatography, high-performance liquid chromatography, and gas chromatography-mass spectrometry. Even though these methods are accurate, they require costly equipments, costly reagents, and trained technicians for the operation of these equipments. The proposed study aims to develop and validate a method which overcomes these limitations.
was sonicated and filtered through 0.4 µ nylon filter using a vacuum pump. Tramadol, being readily soluble in water,\cite{3} gets completely filtered while the particulate matter gets trapped by the nylon filter. A volume of 10 ml filtered drug solution was made up to 100 ml and analyzed.

**Preparation of Eriochrome Black T Solution and Acetate Buffer**

One hundred milligrams of EBT was dissolved in 100 ml DW. To this solution, 25 ml of chloroform was added and stirred well to remove any impurity soluble in chloroform. This was allowed to stand for 10 min to allow the layers to separate. The upper layer of EBT solution was used in the analysis. One gram of anhydrous sodium acetate was dissolved in 230 ml of DW; pH adjusted to 3.5 using diluted glacial acetic acid and made up to 250 ml in a measuring cylinder to obtain the buffer solution.

**Assay Procedure**

To a series of labeled 10 ml volumetric flasks, 250 µl, 500 µl, 750 µl, and 1000 µl of standard solution were added with a micropipette. To each of these, 0.5 ml of acetate buffer and 1.5 ml of EBT solution were added. The drug-buffer-EBT sequence was followed always. The volume was made up to 10 ml with DW. The contents of volumetric flasks were transferred to a series of labeled 100 ml beakers. To each of these beakers, 10 ml of chloroform was also added. The colored aqueous and colorless chloroform layers thus formed were mixed well for 2 min using a magnetic stirrer to let the colored complex move from the aqueous to chloroform layer. After mixing, the contents of the beakers were transferred into a series of labeled separating funnels with glass stopcocks and mounted on stands. The mixed solutions were allowed to stand for 10 min for separation of the aqueous and colored chloroform layers. The latter could be seen in the bottom layer and was drained into labeled 50 ml beakers. The optical density (OD) of these colored chloroform layers was read on a colorimeter with the filter set to 520 nm and using glass cuvettes against a reagent blank treated similarly. Calibration curve was plotted with OD values (Y-axis) against the concentration of the drug (X-axis). OD of the sample solution was also measured in a similar manner and the drug content in the tablet was calculated.

**Method Validation**

The developed method was validated according to the ICH guidelines,\cite{24} in terms of linearity, accuracy, recovery, precision, and robustness. The accuracy of the method was assessed by spiking a sample solution, the mean concentration of which was estimated in the previous step to be 100.8 µg/10 ml, with a known quantity of standard (25 µg added to 10 ml of sample) and the analysis was performed six times. The percentage recovery was then calculated. Precision was determined by intraday variability (repeatability) and interday variability (intermediate-precision) studies. For intraday variability testing, a fixed concentration (100 µg/10 ml) of standard solution was prepared and analyzed six times a day. For interday variability testing, a fixed concentration (100 µg/10 ml) of the standard solution was prepared and analyzed two times a day on 3 consecutive days by different analysts. Robustness, which provides an indication to the reliability of the method, was assessed by making small changes in the method, i.e., changing pH of the buffer by ±0.1 and measuring the effect on OD of a fixed concentration of standard (100 µg/10 ml). Limit of detection (LOD) and limit of

---

**Materials and Methods**

**Instruments and Reagents**

The measurements were made on a Photochem-Micro Digital 8 Filter Colorimeter (Photo Electric Instruments, Jodhpur, India) using glass cuvettes of 1 cm path length. The pure standard of TH was obtained from Sigma-Aldrich, India. EBT was purchased from Spectrum Reagents and Chemicals Pvt, Ltd, Cochin, India. Commercially available tablet dosage forms of TH were used in the study. All glassware used for measuring volumes were Class A. Electronic balance with 0.1 mg sensitivity (Shimadzu, Japan) was used. Eutech CyberScan pH meter was used to measure the pH of buffer solution. Analytical grade chemicals and distilled water (DW) were used. Personal protection equipment used include super nitrile gloves, apron, goggles (3M India), and half face respirator (3M India).

**Preparation of Standard Solution**

One hundred milligrams of TH was dissolved in 100 ml of DW in a volumetric flask and sonicated for 10 min. From this, 10 ml was taken and made up to 100 ml to obtain a standard solution containing 100 µg/ml.

**Preparation of Sample Solution**

Twenty tablets of commercially available TH 100 mg tablets were weighed and powdered. Tablet powder equivalent to one tablet was weighed and dissolved in 100 ml DW. This solution

---

**Figure 1: Structure of tramadol hydrochloride**
quantitation (LOQ) were determined mathematically based on standard error and slope of calibration curve values. LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

**Results**

**Linearity**
The chloroform layer can be seen to take up the colored complex from the aqueous layer after stirring and the intensity of color increased as the concentration of drug increased. This trend was confirmed on measuring the OD [Table 1]. The linearity of the data can be inferred from the calibration curve plotted with the concentration of drug solution on X-axis and OD at 520 nm on Y-axis [Figure 2].

The Beer’s law limits, regression equation, correlation coefficient, LOD, and LOQ were calculated [Table 2]. The linearity of calibration curve can also be appreciated by the high value of correlation coefficient. The mathematically calculated LOD and LOQ values showed that the method is sensitive and that even lower concentrations could be estimated using this method.

**Accuracy and Recovery**
The amount of drug found in the commercial pharmaceutical tablet formulation was found to be in good agreement with the labeled amount [Table 3]. Spiking with 25 µg pure drug yielded a recovery of 99.5% [Table 4]. This indicates that the method is accurate and unaffected by excipients in the tablet.

**Precision**
Intraday precision (repeatability) estimated after six measurements of a fixed concentration of the standard solution on a single day was within 2% relative standard deviation (RSD) [Table 5]. Intermediate precision calculated after estimating six times the drug content in a fixed concentration of the standard solution on different days by different analysts was also within 2% RSD. These results demonstrate the precision of the method and its reproducibility in varying settings.

**Robustness**
Small changes in pH of acetate buffer (±0.1) did not significantly affect the OD readings. This indicates the reliability of the method.

**Discussion**
The principle of this method is that a solution of TH on reaction with EBT in the presence of acetate buffer at pH 3.5 forms an ion-pair complex (colored) which was extracted using chloroform. The OD of this chloroform layer was proportionate to the quantity of TH in the solution. The method used in this study can complete the estimation of TH content in tablet dosage forms in <3 h. All other methods described in the references mentioned above except spectrophotometry and spectrofluorimetry require more than 6 h for completing the estimation (including initialization of equipment and shutting down procedures) and need costly reagents. Since the method involves the use of chloroform, it is essential that proper personal protection equipment (mentioned in materials and methods) are used. Glassware containing chloroform should always be kept closed. The experiment should be done in a spacious and well-ventilated room. The glassware used, especially which come into contact with the colored complex, should be properly washed immediately after the experiment to prevent staining of the glassware. All the steps involved have been well optimized and details provided include the use of glassware and equipment for easy implementation in a health-care provider set-up with minimal investment.

**Conclusion**
The validation parameters discussed above show that the proposed method is reliable, repeatable, reproducible, accurate, and precise.
and is not affected by excipients in tablet formulation. The results also demonstrate that the proposed method is ideal for the estimation of TH in pure drug form and tablet dosage form utilizing minimum resources and time. The added advantage is that it can be performed using a colorimeter which is available in all laboratories and hospitals, thereby facilitating the quality assessment of TH tablets dispensed.

Acknowledgment

We express our heartfelt gratitude to the faculty, nonteaching staff, and junior residents in the Department of Pharmacology, Government Medical College, Kottayam, Kerala, for their whole-hearted cooperation. The authors are thankful to the State Board of Medical Research - Institutional Review Board, Kottayam, Kerala, for funding this study.

Financial Support and Sponsorship

The study was funded by the State Board of Medical Research, Kerala, India.

Conflicts of Interest

There are no conflicts of interest.

References

1. Yaksh TL, Wallace MS. Opioids, Analgesia and Pain Management. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman’s The Pharmacological Basis of Therapeutics. 12th ed. United States: The McGraw-Hill Companies Inc.; 2011. p. 508.
2. Gu J, Qin W, Chen F, Xia Z. Long-term stability of tramadol and ketamine solutions for patient-controlled analgesia delivery. Med Sci Monit 2015;21:2528-34.
3. World Health Organization Expert Committee on Drug Dependence. Tramadol Update Review Report. Geneva; 2014.
4. Rajitha B, Prasanthi S, Reddy RK, Rani TG. Extractive spectrophotometric determination of tramadol hydrochloride in pure and pharmaceutical dosage forms. Int J PharmTech Res 2011;3:114-7.
5. Rathore GS, Basnival PK, Suthar M, Gupta RN. Spectrophotometric estimation of tramadol hydrochloride in pharmaceutical dosage forms. Asian J Chem 2009;21:6111-5.
6. Gogulammudi L, Sujana K. Development and validation of UV spectrophotometric method for determination of tramadol hydrochloride in bulk and formulation. Int J Pharm Pharm Sci 2012;4:275-9.
7. Setty KN, Ramchar T, Chakravarthy IE, Prabhavathi K. A simple spectrophotometric estimation of tramadol hydrochloride in pharmaceutical formulations. Chem Sci Trans 2012;1:317-20.
8. Setty KN, Prabhavathi K, Chakravarthi IE, Gayathri MM. Spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulations. Chem Sci Rev Lett 2013;1:168-71.
9. Srinivasan KK, Alex J, Shirwaiker AA, Jacob S, Sunilkumar MR, Prabhu SL. Simultaneous derivative spectrophotometric estimation of aceclofenac and tramadol with paracetamol in combination solid dosage forms. JIPER 2007;69:540-5.
10. Toral IM, Rivas J, Saldias M, Soto C, Orellana S. Simultaneous determination of acetaminophen and tramadol by second derivative spectrophotometry. J Chin Chem Soc 2008;53:1543-7.
11. Puranik M, Hirudkar A, Wadher SJ, Yeole PG. Development and validation of spectrophotometric methods for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in tablet dosage form. Indian J Pharma Sci 2006;68:737-9.
12. Abdellatif HE. Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation. J Pharm Bioed Anal 2002;29:835-42.
13. Abdellatif HE, El-Henawee MM, El-Sayed HM, Ayad MM. Spectrophotometric and spectrofluorimetric analysis of tramadol, acetabutol and dothiepin in pharmaceutical preparations. Spectrochim Acta A 2006;65:1087-92.
14. Smith AA, Manavalan R, Kannan K, Rajendiran N. Spectrofluorimetric determination of tramadol in formulation and biological fluids. Int J Chem Sci 2008;6:789-99.
15. Padmaja D, Leeja K, Rajpandi R, Babu G. Development of validated spectrofluorimetric method for the quantitative estimation of tramadol hydrochloride in bulk and pharmaceutical dosage form. Int J Appl Pharm Sci Biol Sci 2013;2:101-11.
16. Desai P, Captain A, Kamdar S. Development and validation of HPTLC method for estimation of tramadol hydrochloride in bulk and in capsule dosage form. Int J Pharm Tech Res 2012;4:1261-5.
17. Apshingekar PP, MahadiMV, Dhaneshwar SR. Validated HPTLC method for simultaneous quantitation of paracetamol, tramadol and aceclofenac in tablet formulation. Der Pharm Lett 2010;2:28-36.
18. Ebrahim ZA, Balalau D, Baconi DL, Guttu CM, Ilie M. HPTLC method for the assay of tramadol and pentazocine from mixtures. Farmacia 2011;59:381-7.
19. Kucuk A, Kadioglu Y. Determination of tramadol hydrochloride in ampoule dosage forms by using UV spectrophotometric and HPLC-DAD methods in methanol and water media. Il Farmaco 2005;60:163-9.
20. Kartinasari WF, Palupi T, Indrayanto G. HPLC determination and validation of tramadol hydrochloride in capsules. J Liq Chromatogr Relat Technol 2004;27:737-44.
21. Brahmbhatt KD, Bapna M, Shah SR, Patel RA, Patel CM. Method development and validation of paracetamol and tramadol hydrochloride by RP-HPLC in bulk and pharmaceutical dosage form. Pharma Sci Mon 2013;4 Suppl 1:79-89.

22. Belal T, Awad T, Clark CR. Determination of paracetamol and tramadol hydrochloride in pharmaceutical mixture using HPLC and GC-MS. J Chromatogr Sci 2009;47:849-54.

23. El-Sayed AA, Mohamed KM, Nasser AY, Button J, Holt DW. Simultaneous determination of tramadol, O-desmethyltramadol and N-desmethyltramadol in human urine by gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2013;926:9-15.

24. International Conference on Harmonization, Q2(R1), Validation of Analytical Procedures: Text and Methodology, 2005.