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A Review on Analytical Methods for Estimation of Tramadol Hydrochloride

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ABSTRACT

Tramadol Hydrochloride is an opioid pain medication which is used to treat moderate to moderately severe pain. Various analytical methods used for the estimation of Tramadol hydrochloride has been reviewed in this paper. These include Ultraviolet spectrophotometry, Visible spectrometry, Spectrofluorimetry, High Performance Liquid Chromatography, Ultra Performance Liquid Chromatography, High Performance Thin Layer Chromatography, Gas Chromatography- mass spectrometry, Cyclic Voltammetry, Liquid Chromatography-Electronspray ionization-mass spectrometry, Gas Chromatography, Liquid Chromatography- mass spectrometry, and Capillary Electrophoresis to determine the amount of Tramadol Hydrochloride in bulk drug, pharmaceutical dosage form and biological fluids. Stability indicating methods for Tramadol Hydrochloride are also described. These analytical methods can be used for qualitative and quantitative estimation of Tramadol Hydrochloride in bulk, formulation and biological fluids.

Keywords: Tramadol Hydrochloride, bulk drug, dosage form, biological fluids, analytical methods, estimation.

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INTRODUCTION

Tramadol (marketed as Ultram, and as generics) is an opioid pain medication which is used to treat moderate to moderately severe pain[1].When taken as an immediate-release oral formulation, the onset of pain relief usually occurs within about an hour.[2] Tramadol is chemically known as (1R, 2R)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexan-1-ol[3].

The marketed product of Tramadol is a racemic mixture containing 50% of an R, R-enantiomer ((+) tramadol) and 50% of an S, S -enantiomer ((−)-tramadol). This racemic mixture produces analgesia by synergistic action of its two enantiomers and their O -desmethylated metabolites[4,5,6]. Tramadol is available as drops, capsules and sustained-release formulations for oral use, suppositories for rectal use and solution for intramuscular, intravenous and subcutaneous injection[7].

The analgesic potency of Tramadol is about 10% of that of morphine following parenteral administration. Tramadol provides postoperative pain relief comparable with that of pethidine, and the analgesic efficacy of tramadol can further be improved by combination with a non-opioid analgesic. Tramadol may prove particularly useful in patients with a risk of poor cardiopulmonary function, after surgery of the thorax or upper abdomen and when non-opioid analgesics are contraindicated. Tramadol is an effective and well tolerated agent to reduce pain resulting from trauma, renal or biliary colic and labour, and also for the management of chronic pain of malignant or nonmalignant origin, particularly neuropathic pain. Tramadol appears to produce less constipation and dependence than equi analgesic doses of strong opioids[7].

Tramadol acts as a μ-opioid receptor agonist,[8,9] serotonin reuptake inhibitor and releasing agent,[10,11,12,13] norepinephrine reuptake inhibitor,[9] NMDA receptor antagonist (IC_{50}=16.5 μM),[14] 5-HT_{2C} receptor antagonist (EC_{50}=26 nM),[15] $(\alpha7)_5$ nicotinic acetylcholine receptor antagonist,[16] TRPV1 receptor agonist,[17] and M_1 and M_3 muscarinic acetylcholine receptor antagonist[18,19].

After oral administration, Tramadol is rapidly and almost completely absorbed. Sustained-release tablets release the active ingredient over a period of 12 hours, reach peak concentrations after 4.9 hours and have a bioavailability of 87-95% compared with capsules. Tramadol is rapidly distributed in the body; plasma protein binding is about 20%. Tramadol is mainly metabolized by O- and N-demethylation and by conjugation reactions forming glucuronides and sulfates. Tramadol and its metabolites are mainly excreted via the kidneys. The mean elimination half-life is about 6 hours. The O-demethylation of tramadol to M1, the main analgesic effective metabolite, is catalyzed by cytochrome P450 (CYP) 2D6, whereas N-demethylation to M2 is catalyzed by CYP2B6 and CYP3A4[7].

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. Its use is not recommended in women who are breast feeding or those who are at risk of suicide[1].

ANALYTICAL METHODS FOR TRAMADOL HYDROCHLORIDE

Various analytical methods have been reported for the estimation of Tramadol Hydrochloride in bulk drug and pharmaceutical dosage form as well as in biological fluids.

ESTIMATION OF TRAMADOL HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

Spectrometric Methods:

A new, simple, rapid, precise and novel spectrophotometric method has been developed and validated by Patel Mitali et al. for estimation of Tramadol Hydrochloride (TMD). The method involved measurement of AUC at wavelengths at 266 and 279 nm. The solutions of standard and the sample were prepared in water. Beer's law obeyed in concentration range of 5 to 25 µg/ ml and correlation coefficient 0.9991. This method was validated for precision, reproducibility, linearity and accuracy as per ICH guidelines. The method was found to be simple, accurate and precise[20].

Lavanya Gogulamudi and Sanjan K described a simple, accurate, precise and cost effective UV-Spectrophotometric method for the estimation of Tramadol Hydrochloride, an Analgesic, in bulk and pharmaceutical dosage form. The drug was first dissolved in N-N-dimethylformamide and final volume was made up with distilled water. The λ_{max} or the absorption maxima of the drug was found to be 271nm. A linear response was observed in the range of 30- 150µg/ml with the regression coefficient of 0.999. The method was validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Tramadol Hydrochloride in quality control of formulation without interference of the excipients[21].

K. K. Rajasekhar et al. developed a simple and sensitive spectrophotometric method for the estimation of tramadol in bulk as well as capsule dosage forms. Tramadol obeyed Beer's law in a concentration range of 10-150 µg/mL exhibiting maximum absorption at 270 nm. The results have been validated statistically and recovery studies confirmed the accuracy of the proposed method[22].

A simple, precise and accurate UV spectrophotometric method was developed and validated by Smita Nayak et al. for analysis of Tramadol hydrochloride (TH) from bulk drug as well as formulation. The drug was dissolved in a suitable solvent and its absorption pattern over the wavelength of 200-400 nm was studied. The drug exhibited absorption maxima at 272 nm. The calibration curve was plotted and found to obey Beer-Lamberts law in the concentration range of 25-150 μg/ml with regression coefficient of 0.996. The method was successfully validated as per ICH guidelines. The developed method was found to be suitable for routine quality control analysis of Tramadol Hydrochloride from bulk as well as formulation without interference of excipients[23].

Mustafa Sayed et al. developed a simple and economic UV spectrophotometric method for estimation of Tramadol Hydrochloride in bulk and tablet dosage form and validated as per ICH guidelines. Method involved Absorbance maxima method which based on the measurement of absorbance of Tramadol Hydrochloride in methanol: water (60:40 % v/v) at λmax of 271 nm. The developed method was validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines. The proposed method was found to be linear within the conc. range of 30-150μg/ml for Tramadol Hydrochloride. The present methods were found to be simple, linear, precise, accurate and sensitive and can be used for routine quality control analysis for the estimation of Tramadol Hydrochloride in bulk and tablet dosage form[24].

G. Bhagirathi Bai et al. developed and validated a simple, accurate, precise and cost efficient spectrophotometric method has been developed for the estimation of TMD in bulk and its capsules dosage form. The maximum wavelength (λ max) was found to be 271 nm in water. The mean percentage recovery of TMD was found to be in range 99.29-100.88 %. Beers law was obeyed in the concentration range of 10-200 μg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation $y = 0.0043x + 0.0758$ with $R^2 = 0.9997$ was obtained. Validation was performed as ICH guidelines for Linearity, accuracy, precision, LOD and LOQ. The proposed method may be suitable for the analysis of TMD in bulk and capsules formulation for routine quality control purposes[25].

Two rapid, simple, precise and economical methods *viz.*, linear regression equation and standard absorptivity were developed and validated by Garvendra Singh Rathore et al. for estimation of Tramadol Hydrochloride in pharmaceutical dosage forms. The estimation of Tramadol Hydrochloride was carried out at 271 nm. Beer-Lamberts law was obeyed in the concentration range of 50-250μg/mL and standard absorptivity A (1 %, 1 cm) was found to be 58.95dl g^{-1} cm^{-1}.

The molar extinction coefficient (e) for the drug was found to be 1767.61 mol^{-1} cm^{-1}. Both methods were validated for linearity, accuracy, precision and robustness[26].

B. Rajitha et al. developed two simple, precise, and rapid extractive spectrophotometric methods for the estimation of Tramadol Hydrochloride in both pure and pharmaceutical dosage forms. The methods were based on the formation of colored complex by the drug with reagents like erichorme black-T (method-I) and orange-G (method-II) in an acidic buffer. The linearity ranges of tramadol hydrochloride were found to be 2 to 18 µg/mL for method-I and 2.5 to 15 µg/mL for method-II. The ion-associated complex formed was quantitatively extracted under the experimental conditions with chloroform and the absorbances of the organic layers were measured at 506 nm and 486 nm for method-I and method-II, respectively. The correlation coefficient (r^2) for method-I and II were found to be 0.999 and 0.999, respectively. The methods were statistically evaluated and were found to be precise and accurate[27].

K. Nagaraja Setty et al. developed and validated a simple, accurate, rapid and sensitive spectrophotometric method for the determination of Tramadol Hydrochloride in pharmaceutical formulations. The method was based on the formation of chloroform extractable complex of Tramadol Hydrochloride with wool fast blue, which shows absorbance maxima at 590 nm against the reagent blank treated similarly. The method obeys Beer's law in the concentration ranges of 50-250 µg/mL. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (% RSD< 2.0 and S.D. < 2.0) for both accuracy and precision study. High recovery and low % RSD reveals the reliability of the method for quantitative study of the proposed method in tablet formulation. The proposed method is simple, rapid accurate, precise, reproducible and economic and can be used for routine quantitative analysis of Tramadol Hydrochloride in pure and tablet dosage form[28].

A new analytical method has been developed and validated by Sagarbechara et al. for the determination of Tramadol Hydrochloride in bulk and marketed formulations. Tramadol Hydrochloride in presence of acidic medium reacts with excess amount of Chloramine-T and remaining Chloramine-T react with Crystal violet to produce blue colour. The final stock solution was made to produce 100µg/ml with methanol. The λmax was found to be 591 nm for assay. The linearity was found in concentration range of 5-30µg/ml. The correlation coefficient was found 0.999. The regression equation was found as Y= 0.025X + 0.001.The method was validated according to ICH Guidelines[29].

Kanakapura B. Vinay et al. described a simple, rapid and sensitive method 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 pLg/mL. The regression coefficient (r) is calculated to be 0.9998 with molar absorptivity value of 6.3×10^3 L/mol/cm and Sandell sensitivity of 0.0478 fig/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[30].

Nagaraja Setty K et al. developed and validated a simple, accurate, rapid and sensitive spectrophotometric method for the determination of Tramadol Hydrochloride in pharmaceutical dosage forms. In this method 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) was utilized for determination of Tramadol Hydrochloride forming charge transfer complex with maximum absorbance at λmax 455 nm. Obedience to Beer's law permitted the assay of Tramadol Hydrochloride in their dosage form. The proposed method is simple, rapid accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of Tramadol Hydrochloride in pure and tablet dosage form[31].

Abdellatef HE described two simple and sensitive kinetic methods for the determination of Tramadol Hydrochloride. The first method is based upon a kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time at 20 min. The absorbance of the colored manganite ions was measured at 610 nm. The second method is based on the reaction of Tramadol Hydrochloride with 4-chloro-7-nitrobenzofurazan (NBD-Cl) in presence of 0.1 M sodium bicarbonate. The spectrophotometric measurements were recorded by measuring the absorbance at 467 nm, at fixed time at 25 min on thermo stated water bath at 90+/-1 degrees C. All variables affecting the development of the colour have been investigated and the conditions were optimized. The absorbance concentration plots in both methods were rectilinear over the range 5-25 and 50-250 microg ml(-1), for the first and second methods, respectively. The determination of Tramadol Hydrochloride by the fixed concentration and rate constant methods is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable[32].

Two simple, economical, precise and reproducible visible spectrophotometric methods have been developed by Kumar Amit et al. for the estimation of Tramadol Hydrochloride (HCI) in tablet formulation. The developed methods were based on the formation of chloroform extractable complex of Tramadol HCl with bromophenol blue and thymol blue in distilled water. The complex with bromophenol blue showed absorbance maxima at 447.6nm and linearity in the concentration range of 2-10µg/ml (Method I).The complex with thymol blue showed absorbance maxima at 420.8nm and linearity in the concentration range of 10-60µg/ml (Method II). The results of analysis for both the methods were validated statistically and by recovery studies[33].

Ravindra. N developed two simple and sensitive visible spectrophotometric methods have been developed for the quantitative estimation of Tramadol Hydrochloride from its tablet formulation. The developed methods are based on formation of chloroform extractable colored complex of drug with metanil yellow and tropaeolin too. The chloroform extracted complex of drug with metanil yellow showed absorbance maxima at 410.0 nm and linearity was observed in the concentration range of 3-24 µg/ml (method-I), with tropaeolin too showed absorbance maxima at 485.0 nm and linearity was observed in the concentration range of 3-24 µg/ml (method-II). Results of analysis for both the developed methods were validated statistically and by recovery studies[34].

A simple, accurate, sensitive and economic spectrofluorimetric method has been proposed by Deepa Padmaja et al. for the determination of Tramadol Hydrochloride in pure and pharmaceutical dosage form. The developed spectrofluorimetric method for the estimation of Tramadol Hydrochloride is based on the formation of complex between Dragendorff's reagent and Tramadol Hydrochloride, measured at an excitation wavelength of 481nm and emission wavelength of 648 nm. The developed method was statistically validated in terms of validation parameters as per International Conference on Harmonization (ICH) guidelines .The linearity ranges were found to be 1.2 - 2.0 µg/ml with the regression coefficient of 0.999. The limit of detection and limit of quantification were found to be 0.005 and 0.016 µg/ml respectively. Recovery studies were performed in the range 99-101 % to confirm the accuracy of the method. The Precision results, expressed by intra-day and inter-day relative standard deviation values were found to be less than 2% indicating high degree of precision. Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedures as shown by the recovery study via standard addition technique[35].

Chromatographic Methods:

Chromatographic methods like HPLC, HPTLC, UPLC and GC-MS have been reported for the estimation of Tramadol Hydrochloride in bulk drug and pharmaceutical dosage form.

HPLC Methods

A simple, specific, accurate, and precise RP HPLC method has been developed by M. MadhavaRao et al.for the assay of Tramadol HCl in capsule dosage form using C18 column (Hypersil BDS, 250 X 4.6mm, 5.0µm). The sample was analyzed using 29.5 Volumes of Acetonitrile and 79.5 volumes of 0.2%v/v Trifluroacetic acid as a mobile phase at a flow rate of 1.0 ml/min and detection at 270nm. The retention time for Tramadol Hydrochloride was found to be 6.327 min. The developed method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with ICH guidelines. Validation revealed that the method is specific, rapid, precise, reliable, and reproducible. Calibration plots were linear over the concentration ranges 6 – 120mcg/ml. The method can be used for estimation of Tramadol Hydrochloride drug in capsules dosage form[36].

Kumar et al. developed and validated a simple, reproducible high-performance liquid chromatography (HPLC) method for determination of Tramadol Hydrochloride in solid dosage form. The method was achieved on a C18 column (250Ã—4.6 mm, 5.0Î¼m) using 50:50 (v /v) methanol and 0.1% Trifluroacetic acid in water as mobile phase at a flow rate of 1.0 ml/min. UV detection was performed at 220 nm and the retention time of Tramadol Hydrochloride is ~3.3min. The method was validated for specificity, linearity, precision, accuracy and ruggedness. The result of robustness study also indicate that the method is robust and is unaffected by small variation in chromatographic Condition. The method is specific against excipient interference. The linearity of the method was investigated in the concentration ranges from 0.15-0.30 mg/ml, the correlation co-efficient is 0.999 for Tramadol Hydrochloride. Accuracy was between 99.48% and 100.27%, the method was validated as per ICH guidelines. The proposed method was found to be simple, accurate, precise and rapid and could be used for routine analysis[37].

Z Zaheer et al. developed and validated a simple, sensitive, precise & stability indicating RP-HPLC method for the determination of Tramadol hydrochloride in tablet dosage form. The chromatographic conditions comprised of column XTerra® C 18 column (250 mm, id 4.6 mm, 5 µm),Water Ireland using mobile phase Phosphate buffer : Acetonitrile (75:25 v/v) at a flow rate of 1.5 ml/min at an ambient temperature. The retention time of Tramadol was 6.49 min. Tramadol Hydrochloride was subjected to acid & alkali hydrolysis, oxidation, photochemical determination & thermal degradation. The degraded product was well separated from the drug. The linear regression analysis data for the calibration plots showed regression value 0.9999 in the concentration range 200.60-601.80.The value of slope & intercept were 20.87 & -6.87

respectively. The method was validated for precision, recovery, ruggedness & robustness. The drug undergoes degradation under acidic, basic, photochemical & thermal[38].

A simple, specific and accurate high performance liquid chromatographic (HPLC) method for determination of Tramadol in pharmaceutical dosage forms has been developed by Zaghloul et al. Reversed phase chromatography was conducted using μ-Bondapak C18 column (3.9 x 150 nm) with an isocratic mobile phase consisting of 0.005 M triethylamine in 0.01 M Sodium phosphate buffer (pH 5.5) containing 17% Acetonitrile. The effluent was monitored on a UV detector at 230 nm. Each analysis required no longer than 8 minutes. Quantification was achieved by the measurement of the peak-area ratio of the drug to the internal standard (Metoclopramide) and the detection limit was 75ng/mL. Linear response ($r > 0.999$) was observed over the range of 0.1 - 10 μg/mL and was run on 6 different occasions. There was no significant difference ($p < 0.05$) between inter- and intra- day studies for Tramadol determined for two different concentrations (0.5 and 5.0 mg/mL). The mean relative standard deviations (RSD%) of the results of within-day precision and accuracy of the drug was < 7%. The stability of Tramadol at different temperatures indicated that the drug is stable at 4, 25, and 50°C for at least 4 weeks. The effect of light, 1N HCI, and 1 N NaOH on the stability of Tramadol has also been investigated[39].

HPTLC Methods

P. Desai et al. developed performance thin-layer chromatographic (HPTLC) method for quantitative determination of Tramadol HCl. The HPTLC separation was achieved on an aluminium-backed layer of silica gel 60F254 using Ethyl acetate: Methanol: Ammonia(25%) (9.0: 1.0:0.5 ml (v/v/v)) as mobile phase. Quantitation was achieved by densitometric analysis at 271 nm over the concentration range of 1000-6000 ng/spot. The method was found to give compact spot for the drug (R_f 0.76 ± 0.011). The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.9933$. The method was validated for precision, recovery, repeatability, and robustness as per the International Conference on Harmonization guidelines. The minimum detectable amount was found to be 116.38 ng/spot, whereas the limit of quantitation was found to be 352.67 ng/spot. The method was successfully employed for the estimation of Tramadol HCl as a bulk drug and in capsule dosage form[40].

S.N. Meyyanathan et al. developed a new simple, precise, rapid, and selective high-performance thin layer chromatography (HPTLC) method for the analysis of Tramadol in pharmaceutical formulations. The method uses Chlorzoxazone as an internal standard. The stationary phase was silica gel $60F_{254}$ prewashed with methanol; ethyl acetate-methanol ammonia solution 7 + 1 + 0.5 (v/v) was used as mobile phase. Detection and quantification were performed densitometrically at k = 275 nm. The linear range of the analysis was 1.0 –2.5l g and the percentage recovery was 104.6%[41].

The proposed stability indicating method for the determination of Tramadol Hydrochloride by Bhagyashree R. Dhumalet al by high-performance thin-layer chromatography in the pharmaceutical formulations was found to be specific, precise, and validated. The stationary phase employed was a precoated silica gel 60 F_{254} aluminum TLC plate. The mobile phase containing a mixture of Ethyl acetate: Methanol: Ammonia (9:0.8:0.5 v/v/v) was found to be satisfactory for the resolution of degradation products from the parent drug. Densitometric analysis of Tramadol Hydrochloride was carried out in the reflectance–absorbance mode at 271 nm. The R_f value of the drug was obtained at 0.64 ± 0.02 with sharp symmetrical peak. The degraded products formed under acidic, oxidative, and an alkali conditions were strongly retained. Linear relationships between concentration of analyte and corresponding peak area were observed over the range of 1000–6000 ng at the selected wavelength with an R^2 value of 0.999 ± 0.0007. The validation for specificity, precision, robustness, and recovery of the method was also performed. The present method effectively separated the Tramadol Hydrochloride from its degradation products[42].

UPLC Method

Kanakapura B et al. developed and validated a simple, precise and accurate stability-indicating isocratic UPLC method for the determination of Tramadol Hydrochloride (TMH) in bulk drug and in its tablets. The method was developed using Waters Aquity BEH C18 column (100 mm 2.1 mm, 1.7 µm) with mobile phase consisting of a mixture of Potassium dihydrogen phosphate buffer of pH 2.8 and an equal volume of Acetonitrile (60 : 40 v/v). The eluted compound was detected at 226 nm with a UV detector. The standard curve of mean peak area versus concentration showed an excellent linearity over a concentration range 0.5–300 $\mu g\, mL^{-1}$ TMH with regression coefficient (r) value of 0.9999. The limit of detection (S/N) was 0.08 $\mu g\, mL^{-1}$ and the limit of quantification (S/N) was 0.2 $\mu g\, mL^{-1}$. Forced degradation of the bulk sample was conducted an accordance with the ICH guidelines. Acidic, basic, hydrolytic, oxidative, thermal and photolytic degradation were used to assess the stability indicating power of the method. TMH was found to degrade significantly in acidic, basic and oxidative stress conditions and stable in thermal, hydrolytic and photolytic conditions[43].

GC-MS Method

Dispersive liquid–liquid microextraction (DLLME) coupled with gas chromatography–mass spectrometry (GC–MS) has been developed by SaeedHabibollahi et al. for pre-concentration and determination of Tramadol, ((±)-*cis*-2-[(dimethylamino)methyl]-1-(3 methoxyphenyl) cyclohexanol–HCl), in aqueous and biological samples (urine, blood). DLLME is a simple, rapid and efficient method for determination of drugs in aqueous samples. Efficient factors on the DLLME process has defined and optimized for extraction of Tramadol including type of extraction and disperser solvents and their volumes, pH of donor phase, time of extraction and ionic strength of donor phase. Based on the results of this study, under optimal conditions and by using 2-nitro phenol as internal standard, Tramadol was determined by GC–MS, and the figures of merit of this work were evaluated. The enrichment factor, relative recovery and limit of detection were obtained 420, 99.2% and 0.08 μg L^{-1}, respectively. The linear range was between 0.26 and 220.00 μg L^{-1} ($R^2 = 0.9970$). The relative standard deviation for 50.00 μg L^{-1} of Tramadol in aqueous samples by using 2-nitro phenol as IS was 3.6% ($n = 7$)[44].

Others:

AyselKucuk et al. analyzed Tramadol with cyclic voltammetry (CV) method in the concentration range 10-100 μg/mL (0.0336-0.33 mM) with a detection limit 5 μg/mL (0.0168 mM). The developed and validated method was successfully applied to the analysis of commercial ampoules. Recovery values were between 89 and 92 %. Developed electro analytical method in this study was accurate, sensitive, precise and reproducible and could be directly and easily applied to pharmaceutical forms. The reaction mechanism of this compound using cyclic voltammetric measurements at different scan rates was also tried to be explained by the electrochemical behaviour of Tramadol[45].

ESTIMATION OF TRAMADOL HYDROCHLORIDE IN BIOLOGICAL FLUIDS

Various methods have been reported for the estimation of Tramadol Hydrochloride in biological fluids like plasma, serum, urine and saliva.

Plasma:

A rapid and sensitive high performance liquid chromatography (HPLC) method with DAD detection for the determination of Tramadol in Human plasma in two different solvent media was developed by Aysel Kucuk et al. The sample preparation from plasma was made with a simple liquid-liquid extraction procedure using Ethyl acetate. The extraction with Ethyl acetate yielded good response. The recovery values of Tramadol from plasma were found to be 97.7% for methanol medium and 94.7% for water medium. The calibration curves for both media were linear (r > 0.9942 for methanol medium and 0.9917 for water medium) in concentration range of 0.5-40 μg mL^{-1} of Tramadol. The limit of quantification (LOQ) values of the method for plasma samples in methanol and water medium were 0.35 and 0.55 μg mL^{-1},respectively. Limit of detection (LOD) values in methanol and water medium were 0.20 and 0.30 μg mL^{-1}, respectively. The developed method in this study can be directly and easily applied for the determination in both media for Tramadol in human plasma[46].

A reversed-phase high performance liquid chromatography (HPLC) method was established by Qu L et al. to determine the concentration of Tramadol Hydrochloride in human plasma. HPLC instrument was used with the column: 150 mm x 4.6 mm (Li Chrosorb C18, 5 microns). The mobile phase was composed of Phosphate buffer solution (0.02 mol/L, pH = 3.7)-Acetamide (83: 17, V/V). Flow rate was 1.0 ml/min. Detection wavelength was 216 nm. The standard curve equation was Y = 0.03244X + 0.6007. The linear range was 25-800 ng/ml. The minimum detection limit was 10.2 ng/ml[47].

Yeh GC et al. developed a modified high-performance chromatographic method using UV detection for determination of Tramadol concentration in human plasma. Plasma samples were extracted with Ethyl acetate in a one-step liquid-liquid extraction (recovery 88.5+/-2.1%). Analysis of the extract was performed on a reversed-phase LiChrospher 60 RP-select B column with a particle size of 5 microm. The mobile phase consisted of 0.05 M KH_2PO_4 aqueous solution (pH 3.5) and Acetonitrile in a ratio of 90:10 (v/v). Metoprolol was used as the internal standard and UV detection at 225 nm was employed. Accuracy of the assay in the concentration range examined was from 1.3 to 11.9% for the intra-day run and from 1.4 to 8.1% for the inter-day run. The precision of this method varied from 1.2 to 8.7%. The reproducibility of the method was determined to be from 0.8 to 7.2% over the six-day period. A limit of detection was 9 ng/ml at a signal-to-noise ratio of 3. This validated method was then applied to the determination of Tramadol concentrations in healthy volunteers after oral administration of 100 mg of Tramadol in capsules of Painlax and Tramal[48].

Chongliang LIN et al. developed a sensitive and simple liquid chromatography/ electrospray mass spectrometry (LC-ESI-MS) method for determination of Tramadol in rat plasma using one-step protein precipitation. After addition of Ketamine as internal standard (IS), protein precipitation by Acetonitrile was used as sample preparation. Chromatographic separation was achieved on an SB-C18 (2.1 mm × 50 mm, 3.5 μm) column with Methanol-0.1 % Formic acid as mobile phase with gradient elution. Electrospray ionization (ESI) source was applied and operated in positive ion mode; selected ion monitoring (SIM) mode was used to quantification using target fragment ions *m/z* 264.0 for Tramadol and *m/z* 237.8 for the IS. Calibration plots were linear over the range of 5-500 ng/mL for Tramadol in rat plasma. Lower limit of quantification (LLOQ) for Tramadol was 5ng/mL. Mean recovery of Tramadol from plasma was in the range 92.8 %-97.4%. RSD of intra-day and inter-day precision were both less than 10 %. This method is simple and sensitive enough to be used in pharmacokinetic research for determination of Tramadol in rat plasma[49].

Saliva:

Aziz Y. El-Sayed et al. developed and validated a high-performance liquid chromatography–diode array detector method (HPLC-DAD) for determination of Tramadol in human saliva. Samples of saliva were prepared utilizing liquid–liquid extraction with Hexane-ethyl acetate (4:1, v/v). Propranolol was used as an internal standard (IS). The linear range and average recovery of Tramadol were 0.25–4.00 μg/mL and 94.70%, respectively. The intra- and inter-assay precisions at low, intermediate and high concentrations were within 1.40–9.79 % and 0.77–3.98%, respectively. Intra- and inter-assay accuracies were within 98.50–107.13% and 100.44–103.89%, respectively. The proposed method was successfully applied to determine Tramadol in saliva concentrations in three healthy volunteers for 24 hours after administration of 100 mg oral doses of Tramadol.[50]

Serum:

Lintz W, Uragg H described a gas chromatographic-mass spectrometric method for the quantitative determination of Tramadol in human serum, plasma or whole blood samples. The method involves the use of [2H2, 15N] tramadol hydrochloride as an internal standard and chemical ionization with isobutane, employing single-ion monitoring for quantification. It is specific, sensitive and precise, and has high accuracy. The within-run coefficient of variation is about 1% between 25 and 200 ng/ml and 1.8-2.9% at the lowest concentrations tested (6.25 and 12.5 ng/ml). The between-run coefficient of variation increases from 1.6% to 5.2% with decreasing concentration from 200 to 12.5ng/ml. The detection limit was about 4 ng/ml for serum samples of 1ml. The method proved suitable for pharmacokinetic studies. Its high sensitivity allows measurements of serum concentrations for at least 30 h after the single administration of therapeutic doses of Tramadol Hydrochloride[51].

A specific, sensitive and precise method for the determination of Tramadol in human serum was described by R. Becker, W. Lintz. It comprises a three-step extraction procedure and a specific determination by capillary gas chromatography with nitrogen-selective detection, using a homologue of Tramadol as an internal standard. The specificity of the method was checked by gas chromatography—mass spectrometry. Precision parameters were 1.7–5.5% (within-run) and 3.2–5.7% (between-run) in the concentration range 12.5–200 ng/ml. The detection limit was about 3 ng/ml[52].

Caiyun Zhang et al. investigated a capillary electrophoresis (CE) coupled with end-column electrochemi luminescence (ECL) detection method for the analysis of Tramadol (TMD) has been investigated. ECL detection was working electrode biased at 1.2 V in a 20 mmol·L−1 sodium phosphate buffer (pH = 8.0) containing 5 mmol·L−1 $Ru(bpy)^{2+}_3$ (where bpy = 2,2'-bipyridyl). Linear correlation ($r \geq 0.997$) between ECL intensity and drug concentration was obtained in the range 3 × 10−4 - 6 × 10−6 mol·L−1. The limits of detection (LODs) for Tramadol in water was 3.012 × 10−8 mol·L−1 (S/N = 3). The relative standard deviation values on peak size (10−5 mol·L−1 level) and migration time for the Tramadol were 4.58% and 1.39% ($n = 10$), respectively. Applicability of the CE-ECL method to the analysis of human serum spiked with Tramadol was examined[53].

Urine:

Omnia A. Ismaiel, Mervat M. Hosny developed and validated a simple, sensitive and rapid method for the analysis of Tramadol in human urine was developed and validated. The analyte was isolated from basified urine using a single liquid–liquid extraction then analyzed by spectrophotometry after ion-pair formation. The calibration curves were linear in the range of 10 - 50 μg/mL , the limit of quantitation and the limit of detection were calculated. The method was also valid to determine Tramadol at higher concentrations (250 μg/mL) after dilution. The resulting method demonstrated intra and inter-day precision within 6.3 and 9.5%, respectively, and accuracy within ± 9.9 and ± 3.8%, respectively[54].

A developed Dispersive liquid liquid microextraction (DLLME) as binary solvents-based dispersive liquid-liquid microextraction (BS-DLLME) combined with high performance liquid chromatography (HPLC) with fluorescence detection (FD) was employed by Vahid Kiarostami et al. for determination of tramadol in the urine samples. This procedure involves the use of an appropriate mixture of binary extraction solvents (70μL CHCl3 and 30 μL ethyl acetate) and disperser solvent (600 μL acetone) for the formation of cloudy solution in 5 ml urine sample comprising tramadol and NaCl (7.5%, w/v). After centrifuging, the small droplets of extraction solvents were precipitated. In the final step, the HPLC with fluorescence detection was used for determination of Tramadol in the precipitated phase. The detection limit (S/N = 3) and quantification limit (S/N = 10) were found 0.2 and 0.9 μg/L, respectively. The relative standard deviations (RSD) for the extraction of 30 μg L of Tramadol was found 4.1% (n = 6). The relative recoveries of Tramadol from urine samples at spiking levels of 10, 30 and 60 μg/L were in the range of 95.6 – 99.6%. Compared with other methods, this method provides good figures of merit such as good repeatability, high extraction efficiency, short analysis time, simple procedure and can be used as microextraction technique for routine analysis in clinical laboratories[55].

Plasma and Urine:

Liu P et al. developed and validated a sensitive and selective liquid chromatography-tandem mass spectrometric (LC-MS) method for the determination of Tramadol in human plasma and urine. The analyte was separated on a Diamonsil C18 column with ammonium acetate (5 mmol x L (-1))-methanol (50:50,v:v) adjusted PH by caustic soda at a flow rate of 0.8 ml min(-1), and analyzed by mass spectrometry is in positive ion mode. The ion mass spectrum of m/z were 264.1 for Tramadol and 248.0 for Tinidazole (I.S.), respectively. The weighted (1/x2) calibration curve was linear over plasma concentration range 1.00-400.00 ng/ml and urine concentration range 0.01-16.00 microg/ml, with a correlation coefficient (r) of 0.9995 and 0.9997, respectively. The lower limit of quantification in human plasma was 1.00 ng/ml. The inter-and intra-day precisions (CV%) in both plasma and urine were lower than 10%, the mean method accuracies and recoveries from spiked plasma samples at three concentrations ranged from 98.2 to 100.1% and 61.6 to 62.9%, respectively. The developed method was successfully applied to determine Tramadol in human plasma and urine, and provided suitable profiles for clinical pharmacokinetic study of Tramadol[56.]

CONCLUSION

Various analytical methods have been reported for the estimation of Tramadol Hydrochloride in pharmaceutical formulations and biological fluids. UV spectrophotometry, Visible spectrometry, Spectrofluorimetry, HPLC, UPLC , HPTLC, GC-MS and Cyclic Voltammetry are the methods for estimation of Tramadol Hydrochloride in bulk drug and pharmaceutical dosage form while HPLC, LC-ESI-MS, GC-MS, GC, LC-MS, spectrometric and Capillary Electrophoresis methods are used for estimation of Tramadol Hydrochloride in biological fluids. However, the most widely used method for the estimation of Tramadol Hydrochloride in bulk drug and pharmaceutical dosage form is spectrometric method. Stability indicating methods for Tramadol Hydrochloride by HPLC, HPTLC and UPLC has also been reported.

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