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# Development and Validation of HPTLC Method for Estimation of Tramadol HCl in Bulk and in Capsule Dosage Form

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**1. Abstract:** A new, economic, eco friendly and rapid high-performance thin-layer chromatographic (HPTLC) method was developed and validated for quantitative determination of Tramadol HCl. The HPTLC separation was achieved on an aluminium-backed layer of silica gel $60F_{254}$ 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. Statistical analysis of the data revealed that the method is precise, accurate, reproducible, sensitive and selective for the analysis of Tramadol HCl. The method was successfully employed for the estimation of Tramadol HCl as a bulk drug and in capsule dosage form.

**Key words:** Tramadol HCl, HPTLC, Quantitative analysis.

## 2. INTRODUCTION:

Tramadol HCl is a synthetic codeine analogue that is a weak μ-opioid receptor agonist. It is used as an oral non-steroidal anti-inflammatory drug with good analgesic and tolerability profile in various painful conditions. Chemically it is (+/-)-cis-2-[(dimethylamino)methyl]-1-(3- methoxy-phenyl) cyclohexanol[1]. It is official in British Pharmacopoeia[2], European pharmacopoeia 5.0 and Indian Pharmacopoeia 2010 where potentiometric titration is the official method of assay. Several analytical methods such as spectrophotometric[3], HPLC[4] and bioanalytical methods[5-7] have beed developed. The present study describes a simple, sensitive and precise HPTLC method for the estimation of Tramadol hydrochloride in bulk and in capsule dosage form.

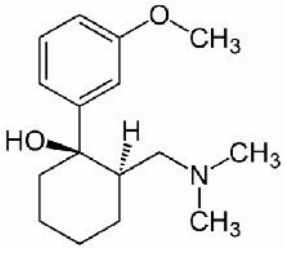


**FIG 1.STRUCTURE OF TRAMADOL HCL**

## 3. EXPERIMENTAL:

### 3.1. Apparatus and reagents

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Limomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (20 × 10 cm), a derivatization chamber, and a plate heater. Precoated silica gel 60 $F_{254}$ TLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were prewashed twice with 10 mL of methanol and activated at $80^0$C for 5 min prior to sample application. Densitometric analysis was carried out using a TLC scanner III with winCATS software. All solvents are of AR Grade and gratis sample of Tramadol HCl was collected from Sun Pharmaceuticals Ltd. And capsule samples were obtained from local market.

### 3.2. HPTLC Method and Chromatographic Conditions

#### 3.2.1. Sample Application

The standard and formulation samples of Tramadol HCl were spotted on Precoated TLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s.

#### 3.2.2. Densitometric Analysis and Quantitation Procedure

Densitometric scanning was performed on Camag TLC scanner III in absorbance mode and operated by winCATS planar chromatography version 1.3.4. The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 271 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. These are selected as recommended by the CAMAG TLC Scanner III manual.

#### 3.2.3. Preparation of Tramadol HCl Standard Stock Solution

Stock solution was prepared by weighing Tramadol HCl (50 mg). Weighed powder was accurately transferred to a volumetric flask of 100 mL and dissolved in and diluted to the mark with methanol to obtain a standard stock solution of Tramadol HCl(500µg/Ml).

#### 3.2.4. Linearity and calibration curve

Linearity of the method was evaluated by constructing calibration curves at six concentration levels.

Calibration curves were plotted over a concentration range of 1000–6000 ng/spot. Aliquots of standard working solutions of Tramadol HCl were applied to the plate (2,4,6,8,10 and 12 µL/spot).The calibration curves were developed by plotting peak area versus concentrations (n=6) with the help of winCATS software.

#### 3.2.5. Validation of developed method

Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness [8].

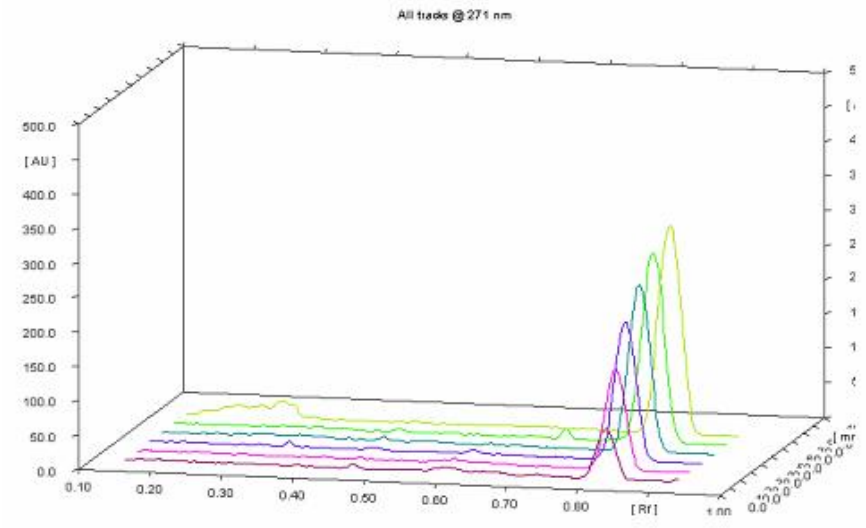


**FIG 2. 3D REPRESENTATION OF DENSITOGRAM FOR CALIBRATION CURVE OF TRAMADOL HCL**

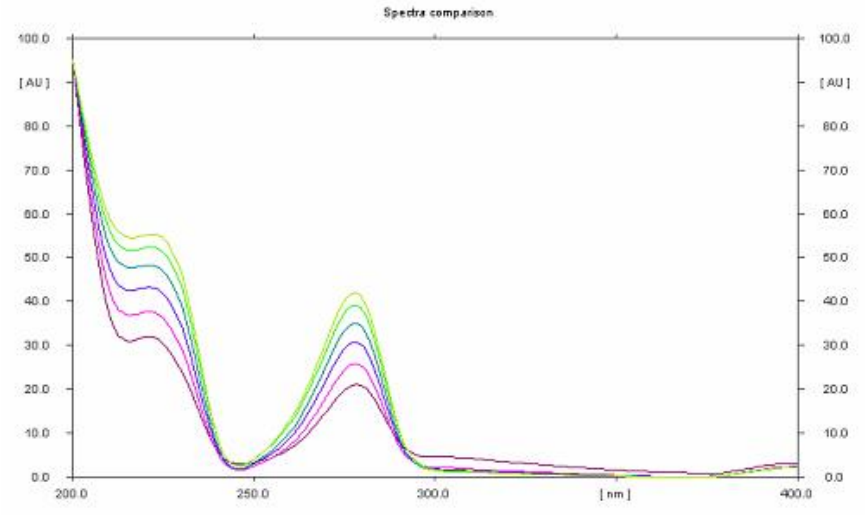


**FIG 3. UV ABSORPTION (REFLECTANCE MODE) OF THE CORRESPONDING SPOTS FOR TRAMADOL HCL**

**TABLE 1. RESULT OF CALIBRATION READING FOR TRAMADOL HCl**

| CONCENTRATION (ng/spot) | AREA MEAN+STADARD DEVIATION(n=4) | RSD |
|---|---|---|
| 1000 | 1647.425 ±15.698 | 0.9529 |
| 2000 | 3099.28 ± 45.0385 | 1.4531 |
| 3000 | 4214.42 ± 15.333 | 0.3838 |
| 4000 | 5403.675 ± 81.930 | 1.5162 |
| 5000 | 6509.175 ± 57.502 | 0.8834 |
| 6000 | 7258.325 ± 18.818 | 0.2592 |

**TABLE 2. STATISTICAL DATA OF TRAMADOL HCl**

| PARAMETERS | RESULT |
|---|---|
| LINEAR RANGE(ng/spot) | 1000-6000 |
| SLOPE | 1.1229 |
| INTERCEPT | 723.75 |
| STDEVIATION OF SLOPE | 0.0058 |
| STDEVIATION OF INTERCEPT | 26.288 |
| LOD(ng/spot) | 116.383 |
| LOQ(ng/spot) | 352.678 |
| CO-RELATION CO-EFFICIENT | 0.9933 |

**TABLE 3. ASSAY RESULT OF MARKETED FORMULATION**

| FORMULATION | ACTUAL CONCENTRATION | %PURITY | LIMIT |
|---|---|---|---|
| CAPSULE | 4037.7 ng/spot | 100.9 ±0.13 | 99%-101% |

**TABLE 4. VALIDATION PARAMETER**

| SUMMARY OF VALIDATION PARAMETER | |
|---|---|
| Recovery(%) | 99.95-100.77 |
| Repeatability(RSD) | 0.7116 |
| Precision(CV)<br>Intra-day(n=3)<br>Inter-day(n=3) | <br>0.0051-0.0078<br>0.0031-0.0326 |
| Specificity | Specific |
| Selectivity | Selective |

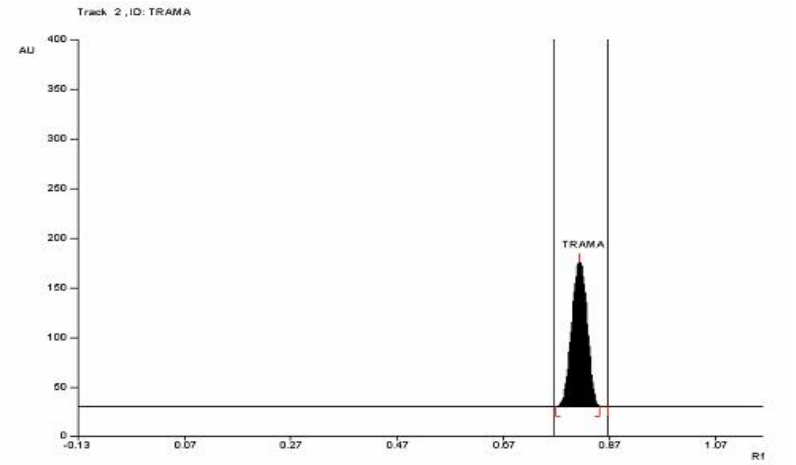


**FIG 4. HPTLC CHROMATOGRAM OF TRAMADOL HCL STANDARD SOLUTION**

## 4. RESULT AND DISCUSSION:

To develop HPTLC method of analysis for Tramadol HCl for routine analysis, selection of mobile phase was carried out on the basis of polarity. A solvent system that would give dense and compact spots with appropriate and significantly different Rf value for Tramadol HCl was desired.Various solvent systems such as acetone-methanol, methanol-chloroform, methanol-toluene, toluene-ethyl acetate, toluene-ethyl acetate-methanol, hexane-ethyl acetate, hexane-acetone, toluene-acetonitrile, and toluene-acetonitrile-glacial acetic acid were evaluated in different proportions. Among these, the solvent system comprising of ethyl acetate-methanol-ammonia (25%) (9.0 - 1.0 - 0.5 v/v/v) gave good separation of Tramadol HCl from its matrix with an *Rf* value of 0.76.

The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application . The method was found to be linear in the range of 1000-6000ng/spot, $y = 1.1229x + 723.75$, $r^2 = 0.9933$ in four replicates. The signal to noise ratios of 3 and 10 were considered as LOD and LOQ respectively. The intraday precision was determined by analyzing standard of drug solution in the concentration range of 2000 ng/spot and 6000 ng/spot for three times on same day while interday precision was determined by analyzing corresponding standards daily for three days over a period of one week.

The specificity of proposed method was confirmed by spotting of marketed formulation and it was observed that the excipients present in the formulation did not interfere with peak of Tramadol HCl.

Recovery studies of the drug were carried out for the accuracy parameters. These studies carried out at three different levels namely 80, 100 and 120%. The result of recovery study indicates the proposed method is accurate for estimation of Tramadol HCl in capsule dosage form.

## CONCLUSION

The proposed method is simple, sensitive, accurate, precise, reproducible, an applicable for the routine estimation of Tramadol HCl in bulk and its pharmaceutical dosage forms.

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