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Research Article

Development and Validation of HPTLC Method for Estimation of Tapentadol Hydrochloride

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ABSTRACT

The main thrust of the paper was to develop and validate a simple, precise high performance thin-layer chromatographic (HPTLC) method for estimation of tapentadol hydrochloride in tablet dosage form. Chromatography was performed on silica gel 60 F_{254} plates with Chloroform: Acetone: Ammonia (2.5: 2.4: 0.1 v/v/v) as mobile phase. This mobile phase system gave a good resolution for tapentadol hydrochloride (R_f value of 0.49 ± 0.02). Detection and quantification were carried out at 272 nm. The linear regression data for the calibration plot showed a good relationship with r=0.999. The limits of detection and quantification were 62.68 and 189.94ng/spot for tapentadol hydrochloride. The amounts of the drugs in the marketed formulation were 99.98%.

Keywords: Tapentadol Hydrochloride, HPTLC Method, Analgesic Effect

INTRODUCTION

Tapentadol hydrochloride(TAP),3-[(1R,2R)-3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol hydrochloride (FIG.1) is a centrally acting analgesic agent that affects the brain and body primarily by activating opioid receptors in the brain, spinal cord and gastrointestinal tract. In addition, Tapentadol inhibits the reuptake of the brain chemical nor epinephrine which possibly has an analgesic effect[1,2]. The drug has dual mode of action in single molecule as an agonist at μ-opioid receptor and as a norepinephrine reuptake inhibitor .With this mode of action Tapentadol provides analgesia at comparable levels of more potent narcotic analgesics such as morphine, oxycodone and hydrocodone[3,4].

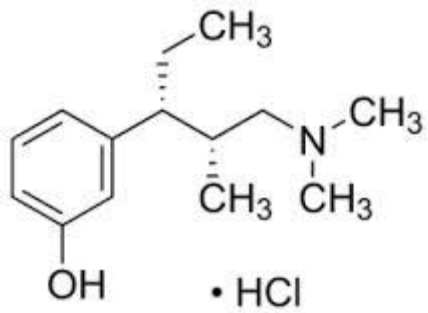

Fig: 1 structural formula of Tapentadol hcl(TAP).

Literature survey reveals that Tapentadol was analyzed by HPLC methods and UV methods in bulk and in formulations.[5,6,7,8] Till now no HPTLC method is developed for quantitative analysis of tapentadol present in literature survey. Hence it was endeavored to develop & validate a simple & precise HPTLC method for estimation of tapentadol.

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PHARMATUTOR
PHARMACY INFOPEDIA
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EXPERIMENTAL

Materials:

Methanol (AR grade), Ethyl Acetate(AR grade), chloroform(AR grade), Acetone (AR grade) ,Toluene(AR grade),Ethyl Acetate(AR grade), Acetic Acid (AR grade), Sodium Hydroxide(AR grade) were supplied by Sisco Research Lab Pvt Ltd, Andheri, Mumbai and Hydrochloric Acid(AR grade) was supplied by S.d. fine-Chem Ltd, Mumbai.

HPTLC instrumentation:

A CAMAG HPTLC system equipped with Linomat 5 Auto-sampler, TLC scanner 4, and winCATS v1.4.7 software (CAMAG, Muttens, Switzerland) was used. The slit dimension was kept at 6.00 mm × 0.1 mm, and 20 mm/sec scanning speed was employed. Chromatography was performed on pre-coated silica gel 60 F_{254} TLC plates (20×20cm^2, catalogue no.:1.05554.0007 cm, (E.Merck, Germany) using Chloroform : Acetone : Ammonia (2.5 : 2.4 : 0.1 v/v/v) as mobile phase. The band length 6 mm and distance between bands 10 mm were kept constant throughout the study. Each concentration was spotted five times on TLC plate. The application speed was 150 nL/sec. Ascending development to a distance of 75 mm was performed on (10x10cm^2, 20x10cm^2, and 20x20cm^2) cm twin through chamber (CAMAG). Chromatograms were evaluated via peak area after scanning in absorbance mode at 272 nm.

Preparation of standard TAP solution:

Accurately weighed 10 mg of TAP was transferred into 10 ml volumetric flask and dissolved in water and diluted up to the mark with water to get a stock solution containing 1 mg/ml of TAP (1000 µg/ml TAP).

Preparation of working standard solution:

1 ml of TAP standard stock solution was diluted to 10 ml with water to get TAP working standard solution containing 100 µg/ml of TAP.

Preparation of sample solution of marketed formulation:

Twenty tablets were weighed and finely powdered. The powdered equivalent to 50 mg of TAP were weighed and transferred to 50 ml volumetric flask. To this, about 20 ml of water was added and sonicated for 15 min. The volume was made up to the mark with water and mixed well. The solution was filtered through Whatman filter paper no. 41 and 1 ml of filtrate was diluted to 10 ml with water to obtain a solution containing 100µg/ml TAP.

Method Validation:

This method was validated for the parameters listed below as per ICH guideline Q2 (R1).

Linearity:

Various dilutions of combined working standard solution were spotted on the TLC plates which cover the range of 700-1400 ng/spot to determine the linearity. Each concentration was spotted five times on TLC plate.

Accuracy (percent of recovery):

80%, 100%, and 120% level of concentration of drug were prepared using reference standard of drug and Placebo. The recovery was found to be 99.56- 100.6% for TAP. The closeness of the result nearly to 100 % assured the accuracy of the developed method for the purpose.

Precision:

The precision is the measure of either the degree of reproducibility or of repeatability of the analytical method under normal operating conditions. It provides an indication of random error; results should be expressed as relative standard deviation or co-efficient of variation.

a) Intraday Precision:

Variation of results within the same day is called Intraday precision. The intra-day precision (% RSD) was determined by analyzing samples at 3 different concentrations for three times on the same day.

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Intraday precision was determined by analyzing standard solutions in the concentration range of 800, 1000, 1200 ng/spot of TAP for 3 times in same day.

b)Inter-Day Precision:

Variation of results within different days is called Inter-day precision. The inter-day precision (% RSD) was determined by analyzing samples at 3 different concentrations for 3 days.

The inter-day precision was determined by analyzing standard solutions in the concentration range of 800, 1000, 1200 ng/spot of TAP at the interval of 3 days.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ were estimated by using following equation.

LOD = 3.3 × (SD/Slope)
LOQ = 10 × (SD/Slope)

Where,

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Specificity and Selectivity:

To determine the selectivity of the method R_f value of TAP standard solution and sample solution were compared and peak shape was observed. The specificity was estimated from the purity of the peak obtained at specific R_f value of the analyte.

Procedure of assay:

Twenty tablets were weighed and finely powdered. The powdered equivalent to 50 mg of TAP were weighed and transferred to 50 ml volumetric flask. To this, about 20 ml of water was added and sonicated for 15 min. The volume was made up to the mark with water and mixed well. The solution was filtered through watmann filter paper no. 41 and 1 ml of filtrate was diluted to 10 ml with water to obtain a solution containing 100µg/ml TAP.

RESULT AND DISCUSSION

Selection of detection wavelength:

The sensitivity of HPTLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study solution of 100 µg/ml of TAP was prepared in water. A solution was filled in the syringe and under nitrogen stream it was applied in form of band of having concentration of 1000 ng/spot of TAP on a single TLC plate at 10 mm from bottom edge using Camag Linomat V semiautomatic spotting device. Plate was developed in ascending mode in twin-trough chamber previously saturated for 20 min using Chloroform: Acetone: Ammonia (2.5:2.4:0.1 v/v/v) up to 75 mm at 25 ± 1°C and dried in air. Developed plate was subjected to densitometric measurements in scanning mode in the UV region of 200 – 400 nm and the overlain spectrum was recorded using Camag TLC Scanner 5. Here, 272 nm is the wavelength for the drug which gives higher absorbance for the drug. Hence 272nm is selected as a detection wavelength.

Method validation:

Linearity:

The linearity for TAP found in the range of 700-1400 ng/spot was shown in chromatogram of TAP. Calibration data of TAP was presented in table. Correlation coefficient, linearity equations (slope and intercept) were presented in Table1.

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Table:1 Result Of Linearity

Parameter for	TAP
Linearity range	700-1400 ng/spot
Linearity equation	y = 2.0451x – 124.02
Correlation co-efficient	0.9994
SD of Slope	0.040
SD of Intercept	38.84

Accuracy (% Recovery):

80%, 100%, and 120% level of concentration of drug were prepared using reference standard of drug and Placebo. The recovery was found to be 99.56- 100.6% for TAP. The closeness of the result nearly to 100 % assured the accuracy of the developed method for the purpose.

Table: 2 Accuracy data of TAP (n=3)

Drug	Amt of std drug added (ng/spot)	%Recovery	%RSD
TAP	800	99.79	1.14
	1000	100.60	0.97
	1200	99.56	1.42

Precision:

Intraday precision:

The data for intraday precision of method were summarized in Table 3. The % RSD was found to be 1.17-1.64 % for TAP which is less than 2 indicated that the method was precise.

Table:3 Intraday precision

Sr No.	Area of TAP	Mean Peak Area ± SD	% RSD
1	800	1498.65 ± 23.85	1.59
2	1000	1962.34 ± 33.25	1.64
3	1200	2385.13 ± 38.86	1.17

Inter day precision:

The data for inter day precision of method were summarized in table4.The %RSD was found to be 1.45-1.78 % for TAP which is less than 2 indicated that the method was precise.

Table:4 inter day precision

Sr No.	Area of TAP	Mean Peak Area ± SD	% RSD
1	800	1484.80 ± 21.53	1.45
2	1000	1935.75 ± 32.85	1.70
3	1200	2345.67 ± 41.86	1.78

Limit of detection and limit of quantification:

Table :5 LOD and LOQ

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ISSN: 2347-7881

The LOD and LOQ values were 62.68 ng/spot and 189.94 ng/spot for TAP. The data shows that the method is sensitive for the determination of tapentadol.

DRUG	LOD (ng/spot)	LOQ (ng/spot)
TAP	62.68	189.94

Specificity and selectivity:

The specificity of the method was ascertained by the R_f values of the drug in standard and as well as in formulation.

The purity of TAP spot was ascertained by comparing the spectra of standard TAP with that of sample spectra at three levels (lower, middle, higher position of the spot). The confirmation of TAP spot was obtained by over laying the spectra of the spot of drug in standard track and corresponding spot in track of sample for spectral comparison.

The result shown in Table 6 indicated that the spot was of TAP, no other sample component was interfering to TAP.

R_f values of TAP

	Standard	Formulation
Rf value of TAP	0.48±0.02	0.49±0.02

Table: 6 Data of Peak Purity

	TAP	
	r (s, m)	r (m, e)
Standard	0.999968	0.999555
Formulation	0.99972	0.999504

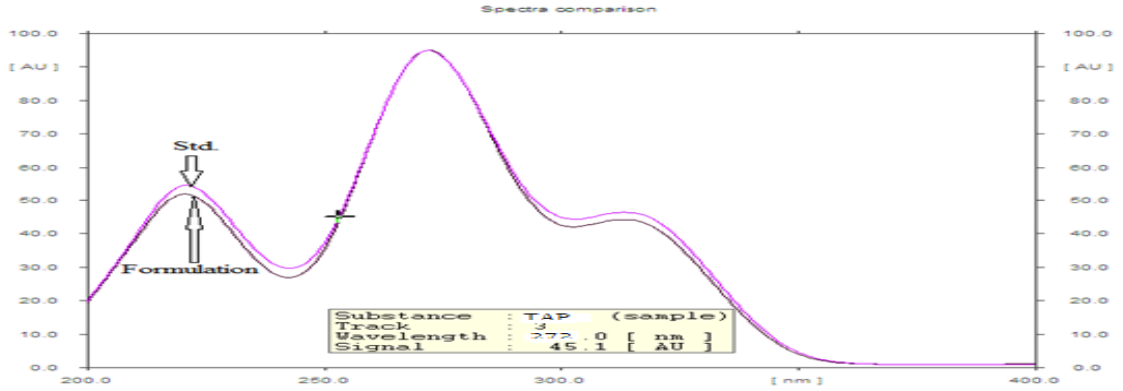

Fig 2 : Comparison of absorbance spectra of standard TAP solution and sample solution.

Application of validated method to pharmaceutical preparation:

Analysis of sample of marketed tablets was carried out by applying 10.0µl of prepared sample solution on HPTLC plate and the amount of TAP present in the sample solution was determined by fitting area values of peak corresponding to TAP into the calibration curves. The results obtained for TAP were compared with the corresponding labeled amounts and recorded in Table 7.

Table:7 Analysis of market formulation (n=3)

Formulation (Tablet)	Label claimed (TAP)	TAP % Assay*±% RSD
VORTH-TP	100 mg/tab	99.98 ± 1.53

CONCLUSION

The HPTLC method for estimation of TAP from pharmaceutical dosage form was developed. The marketed pharmaceutical formulation containing TAP was subjected to quantitative

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analysis using the developed method, yielded nearly 100% assay result for TAP. Hence, it can be concluded that the developed method was sensitive, precise, repeatable and accurate as per ICH guideline and can be used for routine analysis.

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