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Simultaneous Estimation and Validation of Tramadol and Paracetamol by HPTLC in Pure and Pharmaceutical Dosage Form

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A simple rapid, sensitive HPTLC method has been developed and validated for simultaneous estimation of tramadol and paracetamol in pharmaceutical dosage form. It was performed on TLC plate pre-coated with silica getl $60F_{254}$ as a stationary phase using mobile phase comprising of chloroform:methanol:glacial acetic acid (90:20:1) and the detection was carried out of 270 nm showing R_f value 0.48-0.64 for tramadol and 0.68-0.74 for paracetamol. The percentage estimation of labeled claims of tramadol and paracetamol from marketed tablet was found to be 99.9 and 98.95, respectively. The method was validated in terms of accuracy, precision, specificity of ruggedness. Linearity was observed between 50-200 µg/mL for tramadol and paracetamol. The recoveries of drugs by standard addition method were found in the range of 99.86 and 99.88 for both of the drugs. The proposed method is precise, accurate and can be used to estimate the drug contents of marketed formulations.

Key Words: HPTLC, Tramadol, Paracetamol.

INTRODUCTION

Tramadol[1-3] is a non-steroidal antiinflammatory drug. Tramadol is (±) *cis*-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol. Its molecular weight is 299.8. Paracetamol[1-4] belongs to NSAID drugs category. It is chemically N-(4-hydroxyphenyl)acetamide. Its molecular weight is 151.2.

The literature survey[4-10] indicates that the tramadol and paracetamol has been estimated individually by UV, HPTLC and HPLC in pure and pharmaceutical dosage forms. There is no reported method for simultaneous estimation of tramadol and paracetamol. So an attempt has been made to develop a simple, accurate and economical HPTLC method for the simultaneous estimation of tramadol and paracetamol in pure and pharamaceutial dosage forms.

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EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade silica gel $60F_{254}$ precoated aluminum plates with thickness 200 μm, E-Merck, Germany were used as a stationary phase. The instrument used was CAMAG-HPTLC system comprising of CAMAG LINOMAT-IV automatic sample applicator, CAMAG TLC SCANNER III AND VERSIION 4.01 with CAT S 4 software, CAMAG-UV cabinet and CAMAG twin trough glass chamber with stainless steel lids. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190-400 nm. Pure standards of tramadol and paracetamol were obtained as a gift samples from MARAL Laboratories, Chennai, India.

Preparation of standard solution: An accurately weighed quantity of 16.235 mg of paracetamol (RS) and 18.75 mg of tramadol (RS) was dissolved in methanol and made up to 50 mL to obtain a stock solution of 32500 μg/mL of paracetamol and 3750 μg/mL of tramadol.

Mixed standard solution: Solution containing paracetamol and tramadol each of 100 μg/mL was prepared and mixed to get the mixed standard solution.

Chromatographic conditions: Optimized standard chromatographic conditions required were, stationary phase comprising of TLC aluminium foiled plates precoated with silica get $60F_{254}$ with thickness of 200 μm. Chloroform:methanol: glacial acetic acid in the ratio of 90:20:1 v/v/v solution was used as a mobile phase and the chamber was saturated for 10 min. Sample was applied at a constant rate of 0.16 μL/s having scan speed 10 mm/s with 16 mm band width the samples were separated by ascending technique. The chamber was maintained at 20 ± 5 ºC temperature and 50-60 % relative humidity. The scanning was carried out by absorbance/reflectance mode with slit dimension 5 mm × 0.45 mm. The detection was carried out at 270 nm.

System suitability test: The system suitability test was performed by five repeated application for the standard solution containing 2000 μg/mL of tramadol and 1000 μg/mL of paracetamol. The results obtained by repeating the estimation procedure five times were observed to be good.

Standard laboratory mixtures: Different laboratory mixtures were prepared in same manner as that of standard solution to get the final concentration of about 3750 μg/mL of tramadol and 32500 μg/mL of paracetamol. 10 μL of mixed standard solution (duplicate) and laboratory mixture (quadruplet) were applied on TLC plates in the form of 16 mm band. The plates were then developed in pre saturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated densitometrically at a wavelength of 270 nm.

Assay procedure: Twenty tablets were weighed and powdered. An accurately weighed quantity of powder equivalent to 37.5 mg of tramadol and 32.5 mg of paracetamol was transferred to 10 mL volumetric flask. The contents were dissolved in mobile phase and volume made up to the mark. The contents were mixed well using ultrasonicator and filtered through Whatmann filter paper no. 42. This was used as a sample after preparation of the sample the same procedure was followed as under laboratory mixture.

The contents of the drugs in average weight of tablet were calculated as follows:

$$\% \text{ Labeled claim} = \frac{WE}{WA} \times 100$$

where WE = wt. of drug estimated (mg); WA = wt. of drug applied (mg) on the basis of labeled claim.

The results were tabulated in Table-1.

TABLE-1
ESTIMATION OF TRAMADOL AND PARACETAMOL

Drug	Sample	Label claim (mg/mL)	Amount estimated (mg/mL)	Percentage L.C.
Tramadol	1	37.5	37.48	99.90
	2	37.5	37.32	99.52
	3	37.5	37.33	99.71
Paracetamol	1	325	321.60	98.95
	2	325	321.49	98.70
	3	325	321.60	98.95

Validation of proposed method: The proposed method validated for the following parameters.

Accuracy: The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. Accurately known amounts of standard drugs were added to known amount of pre analyzed tablet powder and it was analyzed by the proposed method to ascertain if there are positive or negative interferences from excipients present in formulation. The per cent recovery was calculated by using following formula.

$$\% \text{ Recovery} = \frac{A - B}{C} \times 100$$

where A = total drug estimated in mg; B = amount of drug contributed by tablet powder (as per proposed method); C = amount of pure drug added.

The percentage estimation results are given in Table-1 and the chromatogram of sample formulation are shown in Fig. 1.

Precision: Replicate estimations of drugs in sample were carried out by proposed method and SD/RSD value was calculated as a measure of precision.

Reggedness: Ruggedness was tested under different conditions, *i.e*., analyzing the samples on different days and by different analysts.

RESULTS AND DISCUSSION

Various pure solvents of varying polarity, *viz*., acetonitrile, chloroform, toluene and diethyl ether and their mixtures in different proportions were tried as a mobile phase for development of chromatogram. The mobile phase found to be more suitable was chloroform:methanol:galcial acetic acid 90:20:1 v/v/v. It gave the good resolution

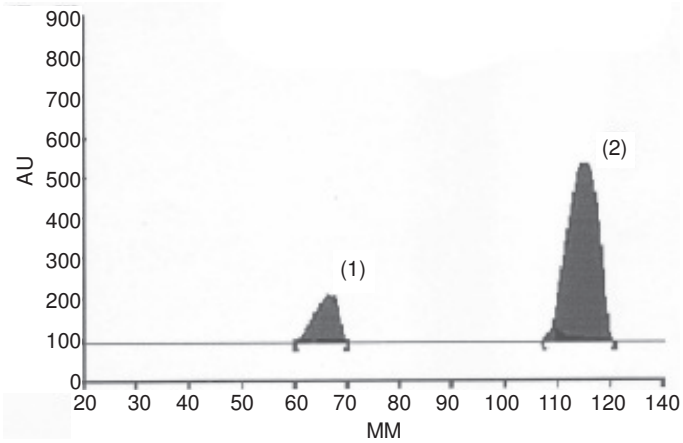

Fig.1. Identification densitogram of mixed standards of tramadol (1) and paracetamol (2)

of two components reasonably good with R_f values of 0.48-0.64 of tramadol and 0.68-0.74 of paracetamol. The 270 nm wavelengths were selected for densitometric evaluation of chromatogram as both drugs have sufficient and high absorbance and showing better sensitivity.

The per cent estimations of drugs in the laboratory mixture with the ± SD were found to be 0.73, 0.857 for the both the drugs and per cent drug estimation in marketed formulation shows 99.86, 99.88 by peak areas for both drugs, respectively.

The concentration response plots of drugs show linearity over the concentration range of 225-525 µg/mL for tramadol and 1950-4550 µg/mL for paracetamol.

The intra-day and inter-day variations of the method were determined using five replicate injections of three different concentrations which were prepared and analyzed on the same day and three different days over a period of 2 weeks, a low coefficient of variation was observed.

The drug content in the tablet was quantified using the proposed analytical method. The system suitability parameters are given in Table-2.

TABLE-2
SYSTEM REPEATABILITY PARAMETERS

Parameter	Tradamol	Paracetamol
Concentration (µg/mL)	15000	100000
Peak area	8723.5	56715.5
Standard deviation	0.073	0.057
% RSD	0.1952	0.0177

To ensure the reliability and accuracy of the proposed method recovery studies were carried out by mixing a known quantity of drug with pre-analyzed sample and contents were reanalyzed by the proposed method. The values are given in Table-3.

TABLE-3
RECOVERY STUDIES

Drug	Amount added (μg/mL)	Amount recovered (μg/mL)	Percentage recovery
Tradamol	3.75	3.70	99.86
Paracetamol	32.50	31.99	99.88

Conclusion

It can be concluded that the proposed HPTLC method is simple, sensitive, rapid and reproducible for the analysis of tramadol and paracetamol in pharmaceutical dosage forms.

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