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Application of TLC- Densitometry Method for Simultaneous Estimation of Tramadol Hcl and Paracetamol in Pharmaceutical Dosage Forms

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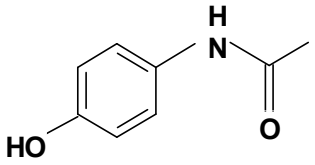
ABSTRACT: A rapid, selective and precise high performance thin layer chromatographic method was developed and validated for simultaneous estimation of Tramadol Hcl and Paracetamol in pharmaceutical dosage forms. The method employed TLC aluminium plates precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of Chloroform: Ethanol (7:3 v/v). This system was found to give compact spots for both Tramadol Hcl (*R*f value of 0.48 ± 0.02) and Paracetamol (*R*f value of 0.85 ± 0.02). Spectrodensitometric scanning-integration was performed at a wavelength of 254 nm. The polynomial regression data for the calibration plots showed good linear relationship with *r*2 = 0.9994 in the concentration range of 2.5 – 32.5µg for tramadol Hcl and 10 – 50µg for Paracetamol with *r*2 = 0.9991. The method was validated for precision, accuracy and recovery. The minimum detectable amounts were found to be 150 ng and 300 ng for tramadol Hcl and paracetamol respectively. The limits of quantification were found to be 450 ng for tramadol Hcl and 900 ng for paracetamol. Statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of tramadol Hcl and paracetamol.
KEYWORDS: Simultaneous Estimation, HPTLC, Tramadol Hcl and Paracetamol.

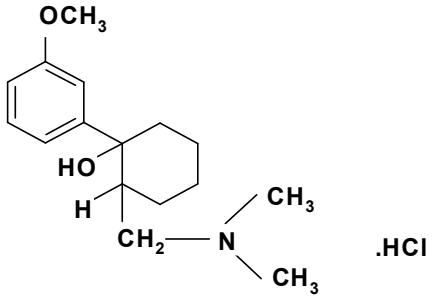
1. INTRODUCTION

Paracetamol, N-(4-hydroxyphenyl) ethanamide (Figure. 4) is a widely used analgesic and antipyretic for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of severe pain (such as postoperative pain) [1]. Tramadol Hcl, (+/-) cis-2-((Dimethylamino) methyl)-1-(3-methoxyphenyl) cyclohexanol hydrochloride (Figure. 5) is a centrally acting analgesic, having agonist actions at the µ-opioid receptor and affects reuptake at the noradrenergic and serotonergic systems. Tramadol is a compound with mild and delayed µ-agonist activity [2]. Literature survey revealed that various analytical methods like spectrophotometric [3-6], HPLC [6-14], GC [15] and HPTLC [16-19] have been reported for the determination of Tramadol Hcl and Paracetamol either individually or combination with some other drugs, but no HPTLC method was reported for simultaneous estimation of Tramadol Hcl and Paracetamol in combined dosage forms. The review of literature prompted us to develop an accurate, selective and precise simultaneous method for the estimation of Tramadol Hcl and Paracetamol in combined dosage forms.

Structure of Paracetamol



Structure of Tramadol Hcl



EXPERIMENTAL

CHEMICALS AND EQUIPMENT

ULTRACET Tablet used for the formulation analysis contains Paracetamol (325 mg) and Tramadol Hcl (37.5mg) and it is manufactured and marketed by Ortho-Mcneil, New jersey. Pure samples were procured from, Tramadol Hcl – Vignesh pharmaceuticals pvt. Ltd, Hyderabad and Paracetamol – Aurobindo pharmaceuticals, Hyderabad. All the chemicals and reagents used were of analytical grade. A Camag HPTLC system comprising of Camag Linomat -5-applicator, Hamilton syringe, Camag twin trough chamber, Camag TLC scanner, and stationary phase pre coated with Silica gel $60F_{254}$ were used.

PREPARATION OF STANDARD SOLUTIONS

The given standard Tramadol 50mg was dissolved in Methanol and made-up to 10ml in a volumetric flask. The given standard Paracetamol 100mg was dissolved in Methanol and made-up to 10ml in a volumetric flask, these solutions were used as working standard solutions for the analysis.

ANALYSIS OF FORMULATION

The given ULTRACET twenty tablets were powdered using Pestle & Mortar to fine powder. From this, 100mg of powdered sample was extracted and dissolved in Methanol, centrifuged and the supernatant liquid was made-up to 10 ml in a volumetric flask with Methanol and filtered through Whattman filter paper no 41. This solution contains 10µg drug sample in 1µl Methanol, used as test solution for quantitative analysis of Paracetamol and Tramadol from ULTRACET tablet. 5µl of the test solution was applied on the pre-coated silica gel $60F_{254}$ plate and from the peak area obtained; the amount of Tramadol Hcl and Paracetamol in formulation was simultaneously calculated using the respective calibration graph. The amount obtained per tablet and percentage label claim are shown in Table 1.

DEVELOPMENT OF CHROMATOGRAMS

The TLC plates were pre washed with methanol and activated by keeping at 115° for about 30 min. The samples were spotted in the form of bands of width 5mm with 100 µl Hamilton syringe on the pre-coated silica gel $60F_{254}$ plate (10×10cm) and the slit dimension was kept at 15 min respectively. The mobile phase used was Chloroform: Ethanol (7: 3 v/v) in chamber and the plate saturation time was 15 min, migration distance was allowed up to 90 mm, linear ascending development was carried out in (20×10cm) twin trough glass chamber. Subsequent to the development, TLC plates were dried in current of air and kept in photo documentation chamber. The images of developed plate were captured at white light, UV 254 nm using Camag – Reprostar -3 instrument. The developed plate was derivatized with iodine vapor and the images were done in white light using Camag – Reprostar -3 instrument. The derivatized plate was scanned at 254nm using Camag-TLC- scanner-3 instrument.

VALIDATION PARAMETERS

LINEARITY

Aliquots of 0.5µl, 2µl, 3.5µl, 5µl & 6.5µl standard Tramadol HCl and a series of 1µl, 2µl, 3µl, 4µl & 5µl standard Paracetamol solution were loaded in the 10 x 10 Silica gel $60F_{254}$ TLC plate using 100µl Hamilton syringe and Camag – Linomat -5 instrument.

LOD AND LOQ

A working standard solution of 150ng/µl and 300ng Tramadol Hcl and Paracetamol were prepared in methanol. Series of 0.5µl, 1µl, 2µl, 3µl, 4µl & 5µl Standard Tramadol Hcl and Paracetamol solutions were loaded in the 10 x 10 Silica gel $60F_{254}$ TLC plate using 100µl Hamilton syringe and Camag – Linomat -5 instrument .

PRECISION

Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days, and percentage relative standard deviation (%RSD) was calculated. The RSD was found to be

less than 2 for both intra-day and inter-day precision. Repeatability of sample application was assessed by spotting 1 µl of drug solution, six times. From the peak areas, the percentage RSD was determined. The complete validation parameters are shown in Table 2.

RECOVERY STUDIES

The recovery study was carried out at three levels, 80%, 100% and 120%. To the powdered formulation, the standard drugs of Tramadol Hcl and Paracetamol were added at 80 %, 100 % and 120% levels, dilutions were made and analyzed by the method. The % recovery and % RSD were calculated and found to be within the limit, as listed in Table 3.

RESULTS AND DISCUSSION

During the stage of method development different mobile phases were tried and the mobile phase comprising of Chloroform: Ethanol (7:3 v/v) was confirmed. A good linear relationship was obtained over the concentration range 2.5- 32.5 µg/spot of Tramadol Hcl and 10-50 µg/spot for Paracetamol respectively. The linear regression data showed a regression coefficient of 0.9994 for Tramadol Hcl (figure.3) and 0.9991 for Paracetamol (figure.2). The LOD with signal/ noise ratio were found to be 150 ng and 300 ng/spot for Tramadol Hcl and Paracetamol respectively. The LOQ with signal/ noise ratio was found to be 450 ng and 900 ng /spot for Tramadol Hcl and Paracetamol respectively. Assay results show excellent label claim of 98.84% for Tramadol Hcl and 99.05% for Paracetamol (table.1). The repeatability showed excellent % RSD less than 0.92 after six applications (table.2). The recovery was 101.8, 101.4 and 102% for Tramadol Hcl and 100.97, 100.67 and 100.87% for Paracetamol at 80% 100% and 120% levels (table.3).

CONCLUSION

The method passes all the validation parameter limits and proves to be selective, sensitive and precise. Hence the developed HPTLC method can be used for the simultaneous analysis of Tramadol Hcl and Paracetamol in bulk drugs and in pharmaceutical dosage forms without any interference from the excipients.

Table -1 RESULT OF ANALYSIS OF FORMULATION

Drug	Amount (mg/tablet)		% label claim*	%R.S.D*
	Labeled	Found*		
Paracetamol	325	323	99.05	0.86
Tramadol Hcl	37.5	37.1	98.84	0.15

*An average value ± relative standard deviation of 5 observations.

TABLE -2 VALIDATION PARAMETERS

Parameters	Value	
	Paracetamol	Tramadol Hcl
R_f	0.85 ± 0.02	0.48 ± 0.02
Linearity (µg/ml)	10 - 50µg	2.5 - 32.5µg
Correlation co efficient	0.9991	0.9994
LOD (ng/spot)	300ng	150ng
LOQ (ng/spot)	900ng	450ng
Precision (% RSD)		
Inter-day	0.82	1.07
Intra-day	0.61	0.72
Repeatability (% RSD)	0.55	0.92

R_f- resolution factor, RSD- relative standard deviation, LOD – limit of detection
LOQ – limit of quantification.

TABLE -3 RECOVERY DATA

Level	Amount added (mg)		Amount found (mg)*		% Recovery*		% RSD*	
	paracetamol	tramadol	paracetamol	tramadol	paracetamol	tramadol	paracetamol	tramadol
80%	260	30	262.54	30.56	100.97	101.8	0.92	1.04
100%	325	37.5	327.24	38.05	100.68	101.4	1.03	1.31
120%	390	45	393.41	45.91	100.87	102	1.07	1.38

*An average value ± relative standard deviation of 5 observations.

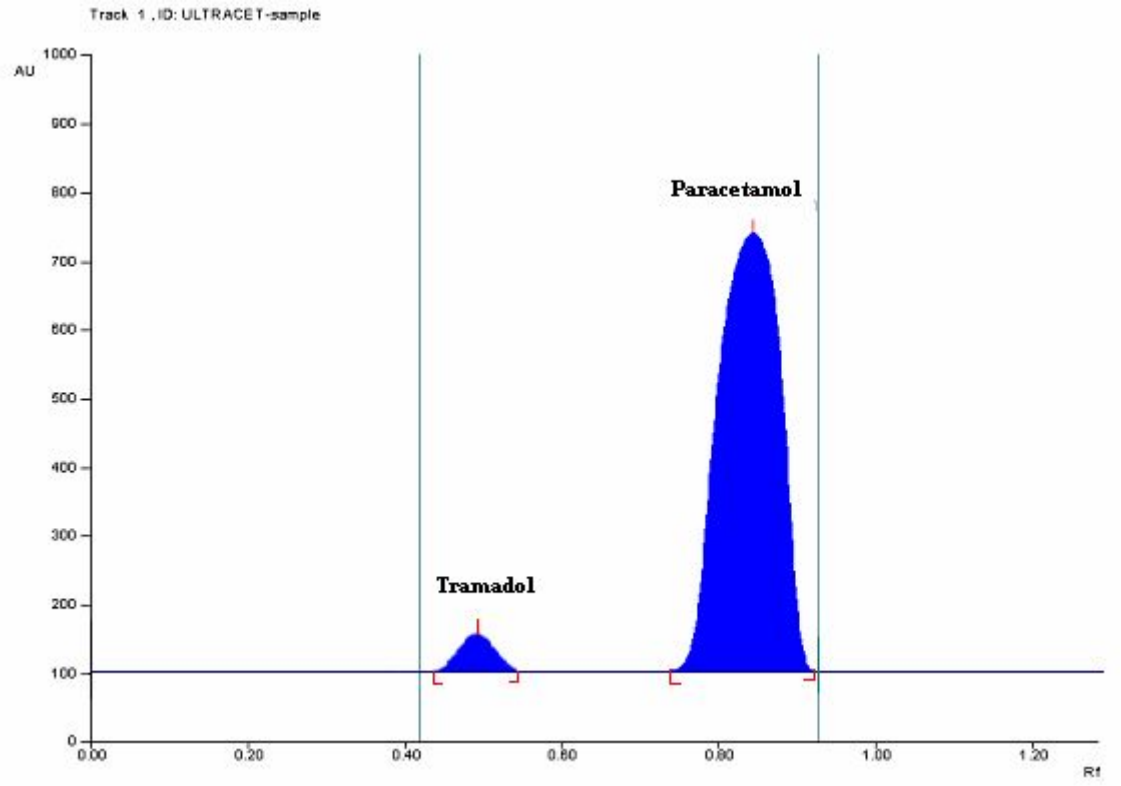


Fig 1: Chromatogram of Tramadol Hcl and Paracetamol.
Chromatogram showing resolution of Tramadol ($R_{f=}$ 0.48) and Paracetamol ($R_{f=}$ 0.85).

Figure.2

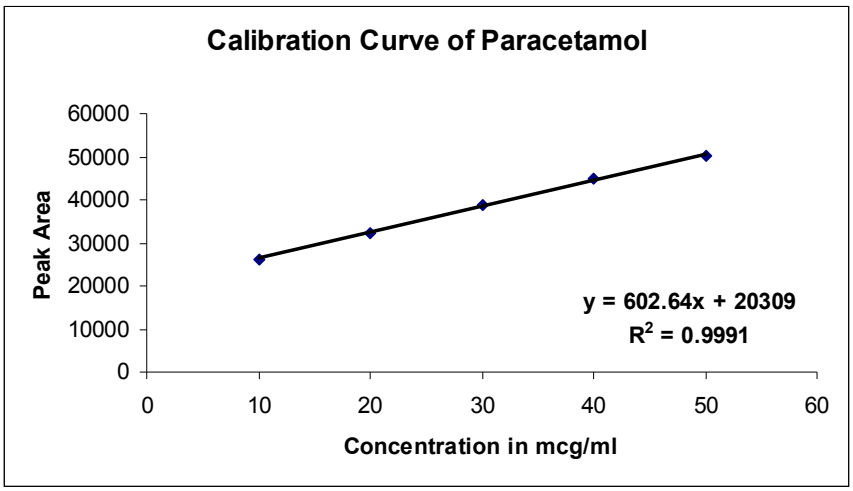
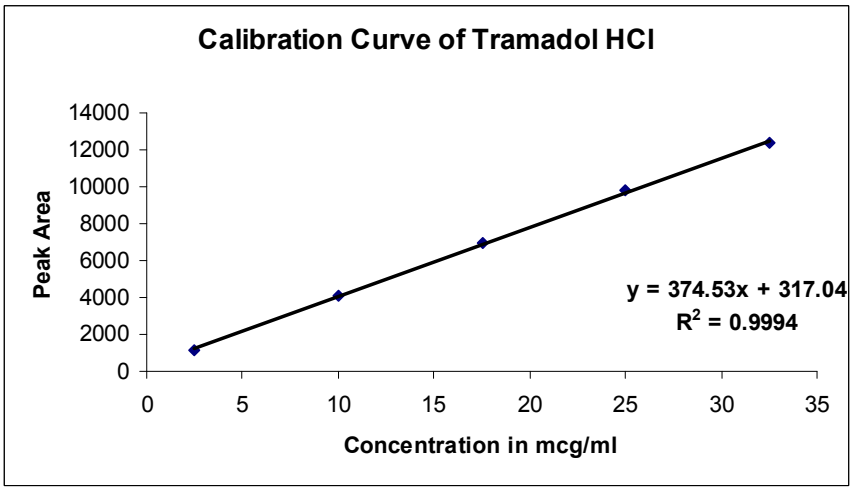


Figure.3



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