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Development and Validation of RP-HPLC Method for Estimation of Tramadol Hydrochloride and Paracetamol in Pharmaceutical Formulation

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***Abstract*:- An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Tramadol Hydrochloride and Paracetamol combined-tablet dosage form. A Enable column (250*4.6mm, 5µm) with mobile phase containing 1% Glacial Acetic acid: ACN(50:50 v/v) was used. The flow rate was 1.0mL/min, column temperature was 30°C and effluents were monitored at 272 nm. The retention times of Tramadol Hydrochloride and Paracetamol were 2.032 min and 2.711 min, respectively. The correlation co-efficient for Tramadol Hydrochloride and Paracetamol was found to be 0.99 and 0.99, respectively .The proposed method was validated with respect to linearity, accuracy, precision, specificity and robustness. Recovery of Tramadol Hydrochloride and Paracetamol formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Tramadol Hydrochloride and Paracetamol in combined dosage form.**

***Keywords:* RP-HPLC, Tramadol Hydrochloride And Paracetamol.**

1. INTRODUCTION

Tramadol hydrochloric (±)-cis-2-(dimethylaminomethyl-1-(3-methoxy-phenyl) cyclohexanol hydrochloride (Figure 1), a synthetic analogue of codeine, is a centrally acting analgesicagent7. It has been used since 1977 for the relief of severe physical pain and has been the most widely sold opioid analgesic drug in the world8.

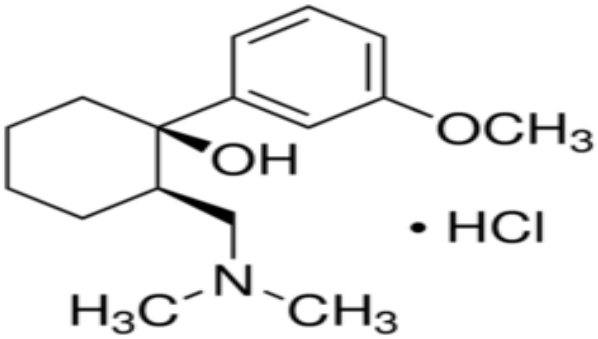

Figure no 1: Tramadol Hydrochloride

The complementary and synergistic actions of two enantiomers improve the analgesic efficacy and tolerability profile of the racemate. Tramadol analgesic effects are also partially reversed by α_2 adrenergic receptor antagonists and the 5-HT_3 receptor antagonist. Tramadol has inhibitory actions on the 5-HT_{2C} receptor.

Paracetamol is chemically 4-hydroxy acetanilide. It is a weak inhibitor of peripheral cyclooxygenase and its analgesic effects mayarise from inhibition of prostanoid synthesis in the CNS. The antipyretic effects ofparacetamol are due to its action at the level of the hypothalamus to reduce pyrogen-initiated alterations in body temperature by inhibiting prostaglandin synthesis5–6.

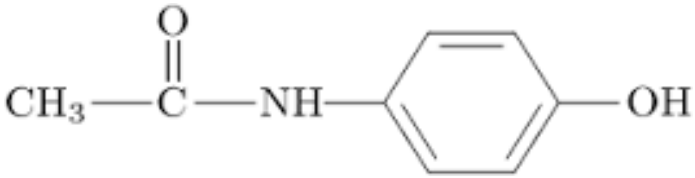

Figure no 2: Paracetamol

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Paracetamol is thought to act primarily in the CNS, increasing the pain threshshold by inhibiting both isoforms of cyclooxygenase, COX-1 and COX-2, enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDS, Paracetmol does not inhibit cyclooxygenase in peripheral tissues and thus has no peripheral anti-inflammatory effects. Paracetmol indirectly blocks COX, and that this blockade is ineffective in the presence of perioxides. The drug selectively blocks a variant of the COX enzyme that is different from the known variant COX-1 and COX-2.

2. EXPERIMENTAL

2.1 Instrumentation. Shimadzu HPLC comprising of LC- 20 AD binary gradient pump, a variable wavelength programmable SPD-20A detector and SCL system controller. A Rheodyne micro-litre syringe fitted with a 20 µl loop was used for injection of sample into the column and data were recorded evaluated by use of LC solutions software.

2.2 Materials. Tramadol and Paracetmol pure samples were procured as gift samples. Calpol T 10 tablets were procured from the local market. Label claim of Calpol T 10 tablets for TRD and Paracetmol were 37.5 mg and 325 mg respectively.

Methanol, Acetonitrile and Glacial acetic acid and water of HPLC grade were purchased from E. Merck and used throughout the experiment.

2.2.1. Mobile Phase Preparation. 1% Glacial acetic acid and Acetonitrile in the ratio of 50:50 v/v was used as mobile phase. The mobile phase was sonicated for 15 min in an ultrasonic bath and filtered through nylon membrane disc filter of 0.45µm pore size using a vacuum pump before pumping into the HPLC system. For the preparation of 1% Glacial acetic acid, 1ml of Glacial acetic acid was taken and dissolved in the 100 ml of HPLC grade water in 100 ml of volumetric flask.

2.3. Chromatographic condition

2.3.1 Chromatographic parameters. Acetonitrile (HPLC grade) and mobile phase consisting of 1% Glacial Acetic acid were filtered through 0.45µ membrane filter prior to use. Before pumping from the solvent reservoir they were degassed. In the ratio of 50:50 v/v were pumped into the column at a flow rate of 1.0ml/min and ambient temperature. The detection was monitored at 272 nm and the runtime was 10min. volume of injection loop was 20µl .prior to injection of the drug solution, the column was equilibrated for about 15min.with the mobile phase flowing through the system.

2.3.2 Preparation of stock standard solution. Tramadol Hydrochloride (100 mg) and Paracetamol (500 mg) were accurately weighed and transferred into 100 ml volumetric flask separately. They were dissolved in 100 ml Acetonitrile to obtain 1000 µg/ml and 5000 µg/ml concentration of stock solutions respectively. From these stock solution 1ml each of TRD and Paracetamol were taken into 10ml volumetric flasks separately and further diluted with a mobile phase to get 100µg/ml and 500µg/ml concentrations of TRD and Paracetamol respectively. The solutions were then filtered through 0.45µm Nylon filter.

2.3.3. Preparation of sample solution. The analysis of drugs, 20 tablets were weighed and triturated in glass mortar and quantity of powder equivalent to 37.5 mg of Tramadol Hydrochloride and 325 mg of Paracetamol was transferred to 100 ml volumetric flask and dissolved in sufficient quantity of Acetonitrile. It was sonicated for 10min and volume was made up to 100 ml to obtain a stock solution of 375 µg/ml of Tramadol Hydrochloride and 3250 µg/ml of Paracetamol. This solution was then filtered through nylon 0.45mm membrane filter. After that 1 ml of the above solution was taken in 10ml volumetric flask and diluted with mobile phase to get 37.5µg/ml and 325µg/ml concentration of Tramadol and Paracetamol respectively. This solution was injected 6 times in to the column and chromatograms were recorded and respective peak areas were measured. The contents of TRD and Paracetamol were calculated by using the regression equation.

2.3.4. Assay of Pharmaceutical Dosage Form. Twenty tablets were taken and their average weight was determined, they were triturated to fine powder. Then powder equivalent to 100 mg of Tramadol Hydrochloride and 11.5 mg of Paracetamol was taken into 100 ml volumetric flask and dissolved in methanol with sonication for 5-10 minutes. The supernatant liquid was transferred into 100 ml volumetric flask through a whatmann filter paper No.41. The residue was washed twice with solvent and combined filtrate was made up to 100 ml mark. After that 0. 5 ml of the above solution was taken in 10ml volumetric flask and was diluted with a mobile phase to get 50 µg/ml and 5.75 µg/ml concentration of Tramadol Hydrochloride and Paracetamol respectively. The assay results for TRD and PARA are shown in the table.

3. RESULTS AND DISCUSSIONS

3.1. Optimization of Chromatographic Conditions. Several HPLC methods were developed for the estimation of Tramadol Hydrochloride and Paracetamol using methanol, water, Acetonitrile and Ortho Phosphoric acid. Hence we have selected 1% Glacial acetic acid and Enable C18G (250 × 4.6 mm i.d.,5µ)column to decrease the retention time and to obtain symmetric peaks having good resolution. Different trails were performed using different proportions of 1% Glacial Acetic acid. The mobile phase containing 1%Glacial Acetic acid and Acetonitrile was found to be satisfactory and gave symmetric and well resolved peak for Tramadol hydrochloride and Paracetamol.

The retention time of Tramadol Hydrochloride and Paracetamol was found to be 2.032 and 2.711. The Theoretical plate count and tailing factor were 4110.026 and 6872.240 and 1.290 and 1.122 for Tramadol Hydrochloride and Paracetamol. The standard chromatogram was shown in Figure 2.

3.2. Validation of Proposed Method. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [19].

3.3. System suitability. System suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. %RSD on five replicate injections of standards

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solution was calculated. The results of system suitability for TRD and Paracetmol are shown in table 1.

3.4. Linearity. Calibration graphs were constructed by plotting peak area Vs concentration for Tramadol and Paracetamol. The calibration graphs were plotted over 15 and 11 different concentrations in the range of 0.5-500 µg/ml and 0.5 – 100 µg/ml for Tramadol and Paracetamol respectively. The regression line obtained was linear. From the data obtained, co-relation coefficient, slope and y-intercept were calculated. Ideally co-relation coefficient should be not less than 0.999 and statistical Y- intercept should be not more than 2.0.

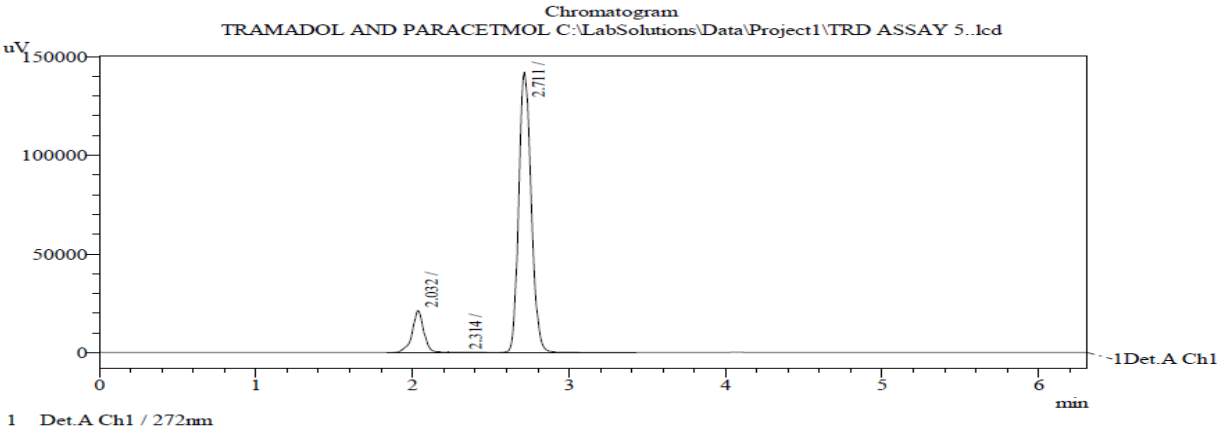

Table1: Chromatographic Characteristics of System Suitability

PARAMETERS	VALUE (Mean ± %RSD)	
	TRD	PARA
Peak Area	142611.5±0.08	3830389±0.003
Tailing Factor	1.251667±0.04	1.123167±0.01
Theoretical Plate	4373.218±0.06	6738.907±0.02
HETP	36.35183±0.01	38.752±0.03
Retention time	2.032	2.711

Table 2: Precision studies of Tramadol Hydrochloride and Paracetamol

Amount of std taken (µg/ml)	Intra-day Precession (n=6)		Inter-day Precession (n=6)	
	Mean±SD	%RSD	Mean ± SD	%RSD
Tramadol Hydrochloride	112602.167±1795.872	1.59	115546±1224.692288	0.88
	226174.7±2690.773	1.18	235937.3±3461.825	1.46
	34604±3132.799	0.90	364566.5±3040.948	0.83
Paracetamol	3145365.17±27786.33	1.05	2151502±23880.28527	1.10
	6459133±30278.66	0.46	6454137±27079.26	0.41
	9362753±16657.17	0.17	9261604±14805.89	0.15

Table 3: Method precision studies of Tramadol Hydrochloride and Paracetamol

Amount of Std taken (µg/ml)	Intermediate precission (n=6)	
	Mean±SD	%RSD
Tramadol Hydrochloride	126504.5±2179.923	1.72
Paracetamol	844950±3053.462	0.36

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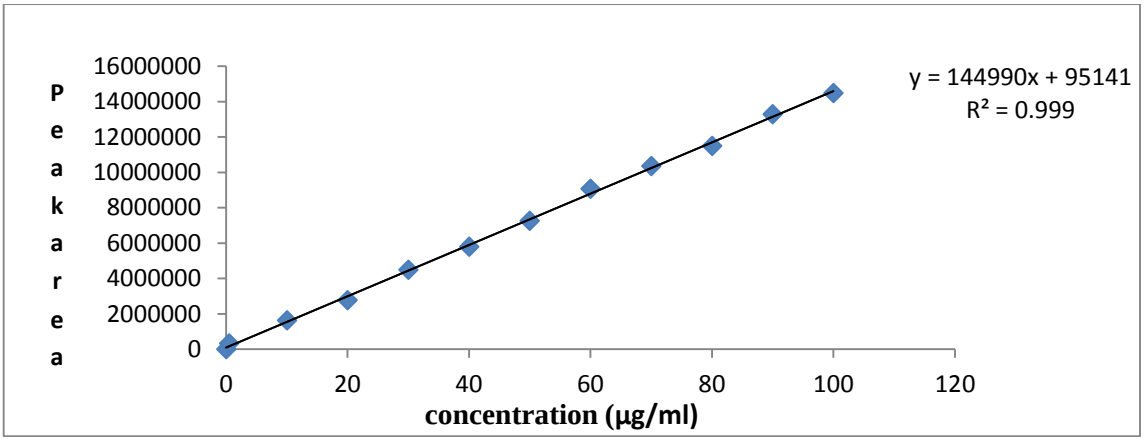

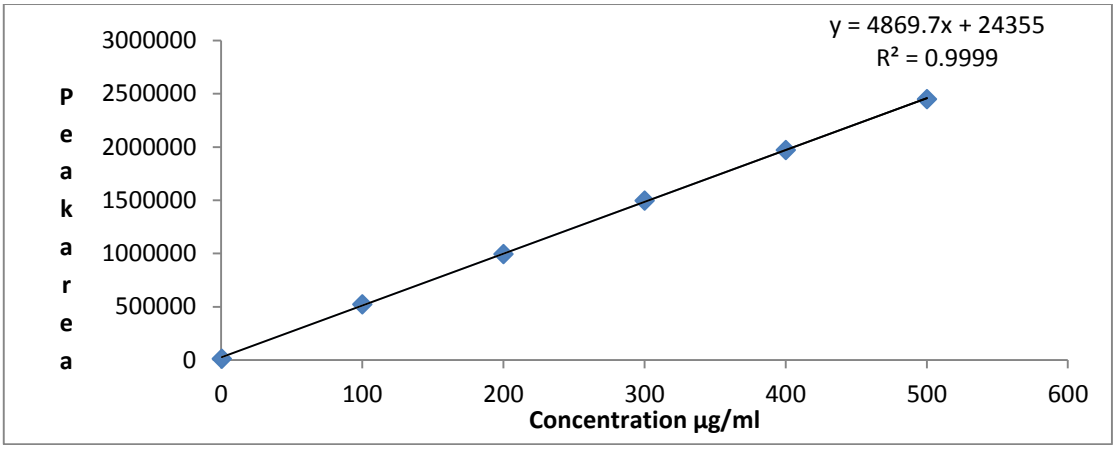

3.4. Precision. Precision was evaluated by injecting six replicate injections of Tramadol Hydrochloride and paracetamol of sample solution under the same chromatographic conditions and calculated by the %RSD. The %RSD indicates that the developed method is repeatable. The %RSD for assay of Tramadol Hydrochloride and Paracetamol was found to be 1.72 and 0.36. The results are shown in Table 2.

3.4.1. Intermediate Precision. The intermediate precision of the method was checked by determining precision on the same instrument, using the same chromatographic conditions in different day. The %RSD of solifenacin was found to be below2 even when it is performed in different day. The method is said to be precise with respect to the criteria of the intermediate precision. The results are given in Table 2.

3.4.2. Method precision (Repeatability): From a homogenous sample solutions are prepared in six replicates and %RSD was determined. The % RSD for six replicate injections shall be not more than 2.0. The results of system precision and method precision for TRD and PARA are shown in the table3.

3.5. Accuracy. In order to judge the quality and applicability of method the recovery analysis was performed at three levels80%, 100%, and 120% by standard addition method. The % recoveries for Tramadol Hydrochloride and Paracetamol were calculated by injecting the samples and it was found to be within the limits; the results are given in Table 4.

3.6. Robustness. The robustness as a measure of method capability to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in mobile phase composition (10% absolute change in organic phase) and flow rate (±1 mL/min) and wavelength. The theoretical plate count and tailing were within the limits. So, the method was found to be robust with respect to variability in all robust conditions.

3.7. LOD and LOQ. The LOD and LOQ of Tramadol Hydrochloride and Paracetamol were determined by using the signal to noise approach as defined in ICH guidelines. The concentration with signal to noise ratio of LOD and LOQ at S/N was 3 and 10, respectively. The results are given in Table 5.

3.8. Ruggedness. Ruggedness of the current method was determined by analyzing six sample solutions of Capol T 325 mg of Paracetamol and 37.5 mg of Tramadol tablet formulation having concentration of 325 $\mu gmL^{-1}$of Paracetamol and 37.5 μgmL^{-1} by two analysts in the same laboratory to check the reproducibility of the test result. The % recovery and standard deviation were calculated in both cases.

3.9. Assay of Pharmaceutical Formulation. The proposed validated method was successfully applied to determine Tramadol Hydrochloride and Paracetamol in its tablet dosage form. The result obtained for Tramadol Hydrochloride and Paracetamol was comparable with the corresponding labelled amounts and they are given in Table 6

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4. CONCLUSION

The present work refers to the fact that the most accurate, precise, and robust HPLC method was developed and validated for estimation of Tramadol Hydrochloride and Paracetamol in pharmaceutical dosage form in accordance with the ICH parameters. The method was validated and found to be simple, rapid, accurate, and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Tramadol Hydrochloride and Paracetamol in its dosage form.

Table4: Accuracy Data

Analyte	%Level	Nominal value (mg)	Found (mg)	%Recovery	Mean% Recovery	%RSD
Paracetamol	80	40	40.02	100.05	100.06	0.05
	100	50	50.02	100.04	100.14	0.20
	120	60	60.11	100.18	100.4	0.37
Tramadol Hydrochloride	80	4.6	4.57	99.34	99.34	0.21
	100	5.75	5.65	99.26	98.37	0.36
	120	6.9	6.87	99.56	99.66	0.17

Table5: LOD and LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Tramadol Hydrochloride	2.462	7.46
Paracetamol	2.95	8.96

Table6: Assay result

S.No	Brand name	Label claim(mg)	Amount taken(mg)	Amount found	%purity ±S.D
1	Paracetamol	325	50	50.13	100.26
2	Tramadol HCL	37.5	5.75	5.71	99.3

Table 7: Robustness

Parameters	Amount to be Detected (Mean ± %RSD)	
	TRD	PCM
Optimised condition (50:50; 1 ml/mim; 272 nm)	115546±1.05	31453565.17±0.88
Change in wave length (271 nm)	114996.8±2.1	2852701±0.38
(273 nm)	114550.8±0.97	2451183±1.61
Change in Mobile Phase 40:60	114357.7±1.1	2655731±0.58
60:50	117739±1.5	2658100±0.35
Change in Flow rate 0.9 ml/min	125232±0.02	2369138±0.79
1.1 ml/min	114631.2±0.02	2470707±0.84

Table 8: Ruggedness

Sample	Analyst - 1	Analyst-2
	(Mean±%RSD)	(Mean±%RSD)
Tramadol HCL	115546±1.05	114620.7±0.96
Paracetamol	6454137±0.41	2641589±0.19

REFERENCES

[1] Sharma B.K, Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut.
[2] Snyder R, KirKland J, Glacjch L, Practical HPLC Method Development, john wiley and sons International publication, II Edn., 2011
[3] International Conference on Harmonization, Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology. Q2 (R1). November 2005.
[4] International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A.1994.
[5] Sohan S. Chitlange, Ramesh D. Bhusal Development and Validation of Spectrophotometric and stability indicating RP-HPLC method for the simultaneous estimation of Tramadol and Paracetamol in tablet dosage form.
[6] LIoyd R.Snyder, Practical HPLC Method Development and Validation for Pharmaceutical Analysis, Mar1, 2004.
[7] Ivana I, Ljiljana Z and Mira Z, J Chromatogr. A, 2006, **1119**, 209□215.
[8] Singh S, Singh B, Bahuguna R, Wadhwal L and Saxena R, J. Pharma Biomed.
[9] Anal., 2006, **41**, 1037□1040**.**
[10] Kumar S.N., Gowda D.G., Mantelingu K and RangappaK.S, Development and validation of hplc method for determination of tramadol hydrochloride in solid dosage form, J. Pharmacy Research, 2012, 5(3), 1438-1440.
[11] Hedpara R, Chhatarala V, Agola.A. et.al (2013). A validated RP-HPLC method for the simultaneous estimation of paracetamol and dicyclomine hydrochloride in pharmaceutical formulation International Journal of pharmaceutical Sciences and research, 4 (6): 2400-2406
[12] Karunakaran K, Navaneethan G and ElangoKP, Development and validation of a stability-indicating rp-hplc method for simulatenous determination of paracetamol, tramadol hcl and domperidone in a combined dosage form, Tropical J.Pharm Research , 2012, 11(1), 99-106.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 11, November - 2017

[13] RAJESH M. KAMBLE and SHRAWAN G. SINGH (2012). Stability – Indicating RP-HPLC Method for analysis of paracetamol and Tramadol in Pharmaceutical Dosage form E-Journal of chemistry 9(3),1347-1356.
[14] Sweetman SC, Ed, In: Martindale: The Complete Drug Reference, 33rd Edn. The Pharmaceutical Press. London, 2002; pp 71,89, 1224.
[15] Kanakapura B.Vinay, Hosakere D. Revanasiddappay, Cijo M.Xavier, et.al(2012). A stability Indicating UPLC method for the determination of Tramadol Hcl : Application to pharamaceutical Analysis Chromatography International research volume 2012.
[16] W. F. Kartinasari, T. Palupi, and G. Indrayanto, "HPLC determination and validation of tramadol hydrochloride in capsules," Journal of Liquid Chromatography and Related Technologies, vol. 27, no. 4, pp. 737–744, 2004.
[17] I. Y. Zaghloul and M. A. Radwan, "High performance liquid chromatographic determination of tramadol in pharmaceutical dosage forms," Journal of Liquid Chromatography and Related Technologies, vol. 20, no. 5, pp. 779–787, 1997.