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[Research article]

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Paracetamol and Tramadol Hydrochloride in Tablet Dosage Form

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.

ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Paracetamol and Tramadol hydrochloride in tablet dosage form. Chromatographic analysis was performed on a Symmetry Thermo C_{18}(150X4.6 mm,5µm) column ambient temperature with a mixture of mixed phosphate buffer and Acetonitrile in the ratio 60:40 (mixed phosphate buffer preparation; 0.01 M Potassium dihydrogen orthophosphate, pH 3.5 adjust with triethylamine) as mobile phase, at a flow rate of 0.80 mL min^{-1}. UV detection was performed at 268 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Paracetamol and Tramadol hydrochloride were 2.250 and 3.378 min, respectively. Calibration plots were linear over the concentration ranges 62.500-375.000 µg mL^{-1} and 6.25-37.50 µg mL^{-1} for Paracetamol and Tramadol hydrochloride respectively. The Limit of detection was 1.8704 and 1.254 µg mL^{-1} and the quantification limit was 5.6679 µg mL^{-1} and 3.8008 µg mL^{-1} for Paracetamol and Tramadol hydrochloride respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.56% to 100.55%.

Keywords: Paracetamol, , Tramadol hydrochloride, RP-HPLC,Validation.

INTRODUCTION

In recent times there has been an increased tendency toward development of stabilityindicating assays1– 3, using the approach to stress testing enshrined in International conference on harmonization (ICH) guideline Q1A (R2)[4]. This approach is being extended to drug combinations to enable accurate and precise quantification of several drugs in the presence of their degradation products. Paracetamol is chemically 4-hydroxy acetanilide (Figure 1). It is a weak inhibitor of peripheral cyclooxygenase and its analgesic effects may arise from inhibition of prostanoid synthesis in the CNS. The antipyretic effects of paracetamol are due to its action at the level of the hypothalamus to reduce pyrogen-initiated alterations in body temperature by inhibiting prostaglandin synthesis[5 - 6.] Tramadol hydrochloric (±)-cis-2-(dimethylamino) methyl-1- (3-methoxy-phenyl) cyclohexanol hydro chloride (Figure 1), a synthetic analogue of codeine, is a centrally acting analgesic agent[7]. 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. There are

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many reported methods for analysis of tramadol[9-13] or paracetamol[14-17] either alone or in combination with other drugs18-20 in pharmaceutical dosage forms. Very few reports are there on simultaneous estimation of paracetamol and tramadol. They were determined in human plasma samples using liquid chromatography (LC-MS)[21-22]. In tablets they were estimated using spectrophotometry[23-24,] HPTLC[25-26], GC-MS27 and HPLC[27-30] methods. Till date, to the best of our knowledge, Two method has been reported in the literature. This manuscript describes the development and validation, in accordance with ICH guidelines, of rapid, economical, precise and accurate isocratic reversed-phase HPLC method for analysis of paracetamol and tramadolHCl in table dosage form

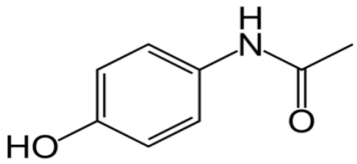


Figure-1 Paracetamol

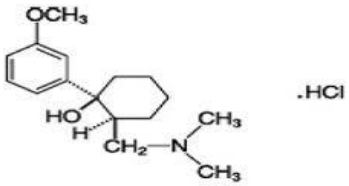


Figure-2 Tramadol HCl

MATERIALS AND METHODS

Chemicals

paracetamol and tramadolHCl obtained from Bio Leo.lab.Pvt.Ltd, Hyderabad, as a gift samples. Potassium dihydrogen orthophosphate & Dipotassium hydrogen orthophosphate (AR Grade), ortho-phosphoric acid (AR Grade), Acetonitrile (HPLC Grade), were purchased from Merck (India) Ltd., Worli, Mumbai, India. Tablet formulation (Altracet) was purchased from local market, containing par acetamol (325 mg), tramadol HCl(37.5 mg). Double distilled water was used throughout the experiment.

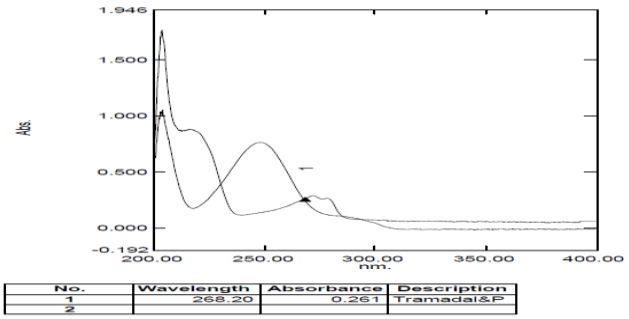
Instruments

Waters HPLC 2 2695 series consisting 4 pump. Auto sampler with 5 racks, each rack has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature.

Waters (alliance) HPLC System is equipped with Empower-2 software.

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions



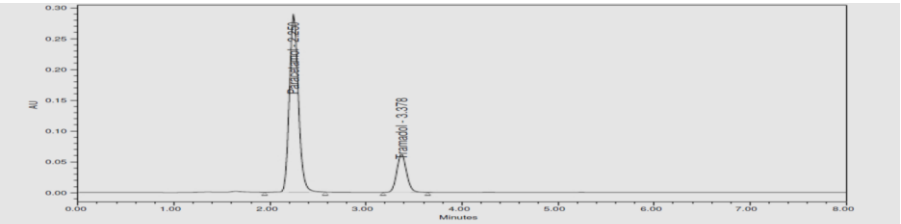
No.	Wavelength	Absorbance	Description
1	268.20	0.261	Tramadal&P
2			

Figure-3 Isobestic point of paracetamol and tramadol HCl

Chromatographic Conditions

A waters symmetry C-18 column (150 mm x 4.6 mm i.d., 5-µm) was used for chromatographic separation. The mobile phase composed of Acetonitrile and mixed phosphate buffer (60:40 *v/v*); pH adjusted to 3.5 with trietalamine at a flow rate of 0.8 mL min-1 with run time of 8min. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 268 nm.

Figure-4 Optimized Chromatogram



	Peak Name	RT	Area	Height	% Area	USP Tailing	USP Plate Count	USP Resolution
1	Paracetamol	2.250	1876422	287562	81.18	1.14	3621	
2	Tramadol	3.378	434822	61940	18.82	1.10	5322	6.24

Fig: 4 Optimized Chromotogram

METHODOLOGY

Mobile phase preparation

Buffer preparation

0.01 M Potassium dihydrogen orthophosphate adjust pH to 3.5 with triethylamine.

Mix buffer and Acetonitrile at 60 : 40 ratio sonicate the resulting solution and degass it using vacuum filtration through 0.4 micron membrane filter.

Standard stock solution preparation

Weigh and transfer 500 mg of Paracetamol working standard and 50 mg of Tramadol working standard into 200 mL volumetric flask, add 50 mL of diluent and sonicate to dissolve and dilute to volume with diluent.

Standard preparation

Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

Sample Preparation

Finely grind pre weighed 20 tablets. Transfer grinded sample quantitatively equivalent to 500 mg of Paracetamol and 50 mg of Tramadol in to 200 mL volumetric flask add 50 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, placebo solution, Standard solution, Disregard peaks due to blank and placebo if any.

VALIDATION OF METHOD

The HPLC method was validated in accordance with ICH guidelines.

Precision

The system precision of the method was verified by six replicate injections of standard solution containing paracetamol and tramadol HCl. The method precision ws carried out the analyte six times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of paracetamol and tramadol HCl.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of paracetamol and tramadol HCl; a known amount of standard drug powder of paracetamol and tramadol HCl were added at 80, 100 and 120 % level.

Specificity and Selectivity

Specificity of the method was determined through study of resolution factor of drug peak from the nearest resolving peak. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was evaluated by making deliberate variations in few method parameters such as variation of wave length; flow rate and change in mobile phase

composition. The robustness of the method was studied for paracetamol and tramadol HCl

RESULTS AND DISCUSSION

Selection of Chromatographic Conditions and Optimization of Mobile Phase

Mobile phase was optimized to separate paracetamol and tramadol HCl using Symmetry C-18 column (150 mm x 4.6 mm i.d., 5µm). Initially, ACN and phosphate buffer in the ratio of (60:40) were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.5 with Triethyle amine, and mobile phase composition (ACN and phosphate buffer in 60:40 % v/v*)* was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.5. The flow rate of the mobile phase was 0.8 mL min-1. Under optimum chromatographic conditions, the retention time for paracetamol and tramadol HCl was found to be 2.250 and 3.378 min, respectively when the detection was carried out at 268 nm. A typical chromatogram of two drugs is shown in (**Figure 3**).

LINEARITY DATA

The Linear detector response for Paracetamol and Tramadol hydrochloride is demonstrated by concentration versus Area. Over the range of 25 to 150% with respect to the target concentration (Dosage).

Table-1 For Peak Area of Paracetamol

%	Conc(mcg)	Area
25	62.500	456678
50	125.000	937710
75	187.500	1390513
100	250.000	1858978
125	312.500	2312992
150	375.000	2781544

Figure- 5 Calibration curve for Paracetamol

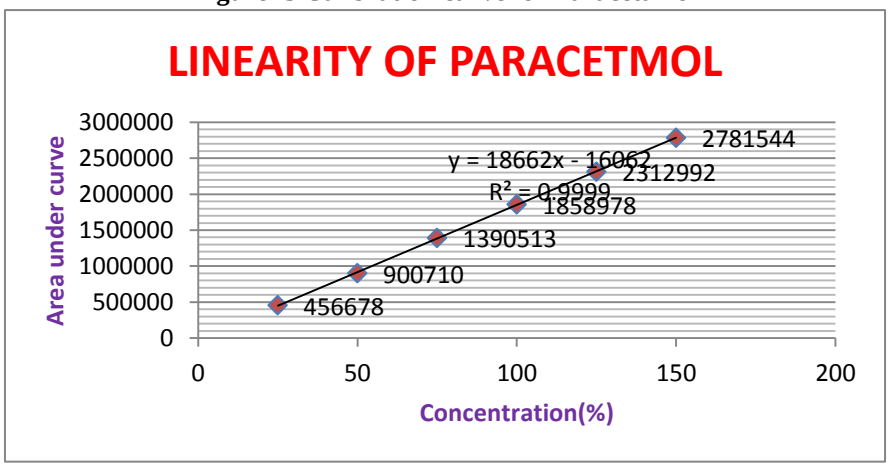


Table-2 For Peak Area of Tramadol hydrochloride

%	Conc(mcg)	Area
25	6.25	106154
50	12.50	217752
75	18.75	322674
100	25.00	431677
125	31.25	537365
150	37.50	647178

Figure-6 Calibration curve for Tramadol hydrochloride

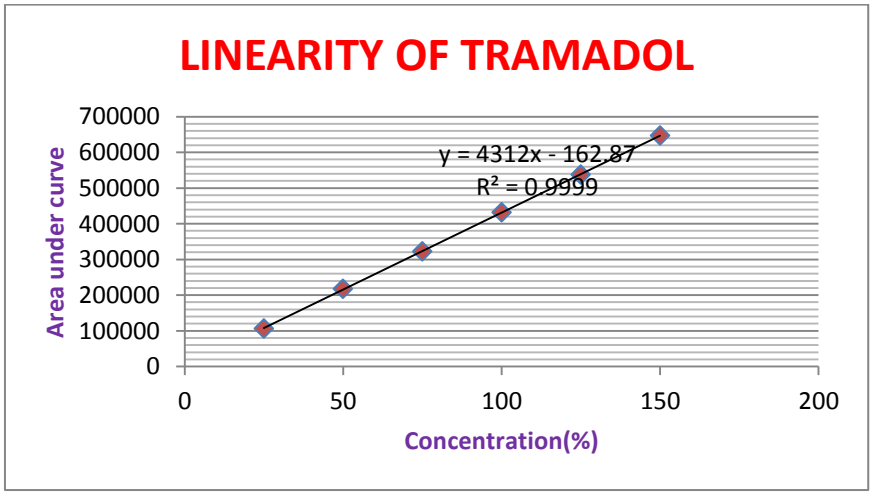


Table-3 PRECISION

S No	Name	Paracetamol		Tramadol HCL	
		RT	Area	RT	Area
1	S-Precision-1	2.251	1851720	3.384	429022
2	S-Precision-2	2.251	1872084	3.378	433645
3	S-Precision-3	2.252	1888269	3.376	436348
4	S-Precision-4	2.251	1876113	3.372	433992
5	S-Precision-5	2.252	1898421	3.371	438659
6	S-Precision-6	2.249	1898764	3.365	438738
Average		2.251	1880895	3.374	435067
Standard Deviation		0.0011	18069.4	0.007	3680.74
RSD		0.0487	0.961	0.19	0.85

Table-4 Method Precision

S No	Name	Paracetamol		Tramadol HC	
		RT	Area	RT	Area
1	M-Precision-1	2.253	1844884	3.381	428605
2	M-Precision-2	2.252	1859494	3.379	431560
3	M-Precision-3	2.251	1880747	3.380	434968
4	M-Precision-4	2.251	1866063	3.376	433370
5	M-Precision-5	2.250	1886423	3.375	437676
6	M-Precision-6	2.252	1885501	3.372	437554
Average		2.252	1870519	3.377	433956
Standard Deviation		0.0010	16616.7	0.003	3536.26
RSD		0.0466	0.888	0.102	0.815

Table-4 System Precision & Method Precision

S No	Name	Paracetamol		Tramadol HCL	
		RT	Area	RT	Area
1	S-Precision-1	2.251	1851720	3.384	429022
2	S-Precision-2	2.251	1872084	3.378	433645
3	S-Precision-3	2.252	1888269	3.376	436348
4	S-Precision-4	2.251	1876113	3.372	433992
5	S-Precision-5	2.252	1898421	3.371	438659
6	S-Precision-6	2.249	1898764	3.365	438738
7	M-Precision-1	2.253	1844884	3.381	428605
8	M-Precision-2	2.252	1859494	3.379	431560
9	M-Precision-3	2.251	1880747	3.380	434968
10	M-Precision-4	2.251	1866063	3.376	433370
11	M-Precision-5	2.250	1886423	3.375	437676
12	M-Precision-6	2.252	1885501	3.372	437554
	Average	2.251	1875707	3.376	434511
	Standard Deviation	0.001	17414.999	0.005	3489.901
	% RSD	0.047	0.928	0.154	0.803

Result

System and Method precision

Paracetamol

% of RSD for RT = 0.047%

Area = 0.928%

Tramadol HCL

% of RSD for RT = 0.154%

Area = 0.803%

Acceptance criteria

The % of RSD for Area and RT from Repeated injections should not be more than 2.0**%.**

ACCURACY

The accuracy of the test method is demonstrated by % of recovery. The sample preparations are spiked with known amount of standard at three concentration levels and injected three times (Like 80% 100% and 120%).

Accuracy data.

Table-5 Standard area

S No	Paracetamol	Tramadol HCL
	Area	Area
1	1858978	431677
2	1855741	431265
Avg	1857360	431471

Table-6 Placebo

S No	Paracetamol	Tramadol HCL
	Area	Area
1	0	0
2	0	0
Avg	0	0

Table -7 Accuracy for Paracetamol and Tramadol hydrochloride

	Accuracy-- 80%		**Accuracy-- 100%**		**Accuracy-- 120%**	
	Paracetamol	**Tramadol HCL**	**Paracetamol**	**Tramadol HCL**	**Paracetamol**	**Tramadol HCL**
S No	**Area**	**Area**	**Area**	**Area**	**Area**	**Area**
Injection-1	1492514	342654	1855965	431655	2204965	520322
Injection-2	1491210	342462	1856045	432115	2217452	520142
Injection-3	1490862	343065	1855748	430856	2234956	521345
Avg area	**1491529**	**342727**	**1855919**	**431542**	**2219124**	**520603**
amt added(mg)	**400.00**	**40.00**	**500.00**	**50.00**	**600.00**	**60.00**
amt Recoverd(mg)	**401.52**	**39.72**	**499.57**	**49.93**	**597.39**	**60.33**
%Recovery	**100.38**	**99.29**	**99.91**	**99.86**	**99.56**	**100.55**

Results (% Of Recovery)

Paracetamol :

At 80% = 100.38 %

At 100% = 99.91 %

At 120% = 99.56 %

Tramadol HCL :

At 80% = 99.29 %

At 100% = 99.86%

At 120% = 100.55 %

Acceptance criteria

The % of recovery should be between 98 to 102%.

LIMIT OF DETECTION (LOD)

Table-8 Limit Of detection results.

S.NO	**Name**	**LOD Value (µg/ml)**
1.	Paracetamol	1.8704
2.	Tramadol hydrochloride	1.254

Table-9 Limit of Quantitation (LOQ)results.

S.NO	**Name**	**LOQ Value(µg/ml)**
1.	Paracetamol	5.6679
2.	Tramadol hydrochloride	3.8008

ROBUSTNESS

Robustness for Paracetamol and Tramadol hydrochloride. The robustness of test method is demonstrated by carrying out intentional method variations like mobile phase flow changes, mobile phase compositions and column oven temperature variations etc...

Table-10 Robustness for Paracetamol and Tramadol hydrochloride

S No		Paracetamol		Tramadol HCL	
		RT	Area	RT	Area
1	Standard	2.251	1875707	3.376	434511
2	Robustness-MP-Flow Change-1	2.036	1708651	3.030	395193
3	Robustness-MP-Flow Change-2	2.517	2145545	3.756	495949
4	Robustness-Column Oven Temperature-1	2.238	1916291	3.570	443878
5	Robustness-Column Oven Temperature-2	2.323	1351173	3.146	181897

The results are mentioned below

Paracetamol

Flow1 = 2.036 min
Flow2 = 2.517 min
Temp-1 = 2.238 min
Temp-2 = 2.323 min

Tramadol HCL

Flow1 = 3.030 min
Flow2 = 3.756 min
Temp-1 = 3.570 min
Temp-2 = 3.146 min

Acceptance criteria

The result should show some variation from standard results.

ASSAY

Assay for Paracetamol and Tramadol hydrochloride

Standard preparation

Transfer 10 ml of standard stock solution in to 100 mL volumetric flask and make up to volume with diluent.

Sample Preparation

Transfer sample quantitatively equivalent to 500 mg of Paracetamol and 50 mg of Tramadol in to 200 mL volumetric flask add 50 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 50 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, standard solution, and sample solution record the chromatogram. And calculate percentage of assay.

Table-11

Paracetamol	500-mg
Tramadol HCL	50-mg
Avg wt	850-mg

Table-12

		Paracetamol		Tramadol HCL	
S No	Name	RT	Area	RT	Area
1	Standard-1	2.252	1869495	3.380	431562
2	Standard-2	2.254	1880747	3.382	434968
Avg		2.253	1875121	3.381	433265
3	Sample-1	2.253	1865425	3.378	430662
4	Sample-2	2.250	1880655	3.381	431265
Avg		2.252	1873040	3.380	430964

Table-13 Results for Paracetamol

1873040	500	10	200	100	99.93	850	**mg/Tab**	**%Assay**
1875121	200	100	850	10	100		**499.45**	**99.89**

Table- 14 Results for Tramadol hydrochloride

430964	50	10	200	100	99.84	850	**mg/tab**	**%Assay**
433265	200	100	850	10	100		**49.73**	**99.47**

Assays result

Paracetamol = 99.89 %

Tramadol HCL = 99.47 %

SYSTEM SUITABILITY PARAMETERS

Table-15 System suitability parameters results for Paracetamol and Tramadol hydrochloride

Parameters	**Results**	
	Paracetamol	**Tramadol hydrochloride**
Tailing factor	1.14	1.10
Theoretical plates per column	3621	5322
Resolution	6.24	

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of Paracetamol and Tramadol hydrochloride in tablet formulation. The method was validated as per ICH guidelines.

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