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Validated RP-HPLC method for simultaneous estimation of paracetamol and tramadol hydrochloride in a commercial tablet

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ABSTRACT

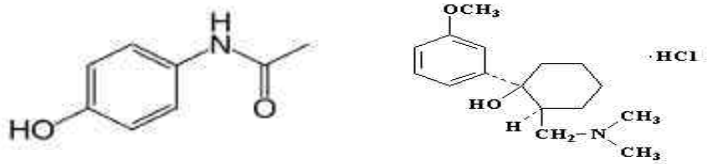
This paper describes development and validation of a high-performance liquid chromatographic method for simultaneous analysis of paracetamol and tramadol hydrochloride in a tablet formulation. The separation of tramadol and paracetamol was achieved on an Inertsil C_{18} reverse phase column (250 x 4.6 mm i.d., 5µm) with methanol: buffer; water adjusted pH 3.4 with ortho-phosphoric acid (40:60, v/v) as mobile phase at a flow rate of 1.0 mL/min. The detection wavelength was set at 228 nm. Under these conditions, separation of the two components was achieved in less than 5.0 min. Analytical characteristics of the separation such as precision, specificity, linear range and reproducibility were evaluated. The developed method was applied for the determination of two drugs paracetamol and tramadol at concentration of 26.0 µL/mL and 3.0 µL/mL respectively. The method was successfully used for determination of both compounds in tablets.

Key word: Paracetamol, Tramadol, Anti-inflammatory drugs, Validation, High- performance liquid chromatography.

INTRODUCTION

Paracetamol (PR), N-(4-Hydroxyphenyl)-acetamide (Fig. 1) is a widely used analgesic and antipyretic for the relief of fever, headaches and other minor aches and pains and it is a major ingredient in numerous cold and flu remedies. In combination with non-steriodal anti-inflammatory drugs (NSAIDs) and opioid analgesic, PR is used also in the management of severe pain (such as postoperative pain)[1]. Tramadol (TR), (1RS, 2RS)-2-[(Dimethyl-amino) methyl]-1-(3-methoxy-phenyl) cyclohexanol hydrochloride (Fig. 1) is a centrally acting analgesic consisting of two enantiomers, both of which contribute to the analgesic activity via different mechanisms[2]. (+)-Tramadol is a synthesic codeine analog that is a weak µ-opioid receptor agonist. It is used as an oral non-steroidal anti- inflammatory drug with good analgesic and tolerability profile in various painful conditions[3]. In literature several methods like spectrophotometry[4-9], HPTLC[10,11] and HPLC[12-22] are reported on simultaneous determination of these two drugs either alone or in their combined doses form along with other drugs.

The simultaneous determination of PR and TR in their combined doses form has been reported in a few publications. They were estimated in human plasma samples using liquid chromatography (LC)-MS[23,24]. In tablets they were determined using spectrophotometry[25,26], HPTLC[27,28], GC-MS[29] and HPLC[29-32] methods. In the reported HPLC methods[29-32] of these two drugs the TR separates after separation of PR within the range of 5 to 6 min retention time. In our developed method TR significantly separates before the separation of PR at retention time between 2.0 to 2.1 minutes and also the two compounds (TR and PR) get eluted within 5.0 minutes using different mobile phase composition; at different concentration range and detected at different wave length. The present work describes the development of a validated RP-HPLC method which can quantify these components simultaneously from a combined dosage form which is fast, simple, precise and reliable method for routine analytical needs as compared to the early reported HPLC methods. The present RP-HPLC method was validated following the ICH guidelines[33,34].



Paracetamol **Tramadol HCl**

Fig.1. Structure of paracetamol and tramadol hydrochloride

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Experimental procedure

Standards and Chemicals:

Paracetamol (standard, Sigma Aldrich), Tramadol (standard, Sigma Aldrich), Water (HPLC grade, Merck), Methanol (HPLC grade, Merck), Ortho-Phosphoric acid (AR grade, Merck) were used throughout the experiment.

Instrumentation:

Chromatographic separations were performed with Thermo Electron Corporation Spectra System having pump series P-2000 HPLC gradiant pump and detector UV/VIS, series UV-1000, a manual injector with a 20 µL fixed loop. The separation was performed on Intertsil ODS C_{18} column (Length: 250 mm, Diameter: 4.6 mm, Particle size: 5 µm). Analyses were carried out at an ambient temperature. An ultrasonicator was used for degassing the mobile phase.

Chromatographic conditions for validation:

Stationary phase: Intersil C_{18} column (Length: 250 mm, Diameter: 4.6 mm, Particle size: 5 µm), Mobile phase: Methanol: buffer; water adjusted pH 3.4 with ortho- phosphoric acid (40:60) v/v, Flow rate (mL/min) 1.0, Run time (min): 8.0, Column temperature (°C): ambient, Injection volume: 20 µL, Wavelength: 228 nm, Retention time: approximate 2.1 min for TR and 3.9 min for PR.

Preparation of stock solution:

Stock solution of PR (650 µg/mL) and TR (75 µg/mL) was prepared by dissolving 65 mg of PR in 100 mL volumetric flask and 7.5 mg of TR in 100 mL volumetric flask with methanol.

Preparation of standard solution:

Standard solution of 26.0 µg/mL and 3.0 µg/mL of PR and TR was prepared respectively by dissolving 4.0 mL from both stock solutions was added to 100 mL volumetric flask and diluted up to the volume with mobile phase.

Preparation of sample solution:

Twenty tablets, each containing 325 mg of PR and 37.5 mg of TR were weighed and finely powdered. A quantity of powder equivalent to 65 mg of PR and 7.5 mg of TR was weighed accurately and transferred to a 100 mL volumetric flask and volume was made up with the methanol and it was filtered using 0.45µm membrane filter. From the above prepared solution 4.0 mL is taken and diluted to 100 mL with mobile phase to give test solution containing 26.0 and 3.0 µg/ml PR and TR respectively. The 20 µL of the solution is injected into the column and chromatogram was recorded.

Validation of proposed method

The assay of two samples PR and TR was validated with respect to linearity, precision, accuracy, robustness and stability.

Accuracy (% recovery):

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method of PR and TR at 80, 100, 120 and 150% level. Known amounts of standard solutions of PR (20.8, 26.0, 31.2 and 39.0 µg/mL)

and TR (2.4, 3.0, 3.6 and 4.5µg/mL) were spiked to prequantified sample solutions. The amount of PR and TR were estimated by applying these values to the regression equations of the calibration curves.

Method precision (repeatability):
The instrumental precision was checked by repeatedly injecting (n = 6) solution containing PR (26.0 µg/mL) and TR (3.0 µg/mL).

Intermediate precision (reproducibility):
The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 6 times on the same day and 6 times on another days for same concentrations of PR (26.0 µg/mL) and TR (3.0 µg/mL). The results are reported in terms of relative standard deviation (RSD).

System suitability test:
In the system suitability test of solution containing 3.0 µg/mL of TR and 26.0 µg/mL of PR were prepared and injected (n = 6). The standard and sample solution were stored at room temperature and analyzed over the time period of initial, 12 hrs and 24 hrs. Then the system suitability parameters like retention time, theoretical plates, tailing factor and resolution were calculated from the chromatogram. Thus absolute difference between % assay values was not more than ± 2.0% compared to the initial value.

Calibration curve (Linearity):
Accurately measured aliquot of working standard equivalent to 20.8 - 39.0 µg/mL of PR and 2.4 - 4.5 µg/mL of TR was transferred to 2 series of 100 ml volumetric flask and the content of the flask were diluted to the volume with mobile phase. A 20 µL aliquot of each solution was injected in triplicate. The conditions including the flow rate of mobile phase at 1.0 mL/min, detection at 228 nm and run time program for about 8.0 min, were adjusted. A calibration curve for each sample was obtained by plotting area response versus concentration which gave a straight line corresponding to the equation: y = mx + c as shown in Figure 2.

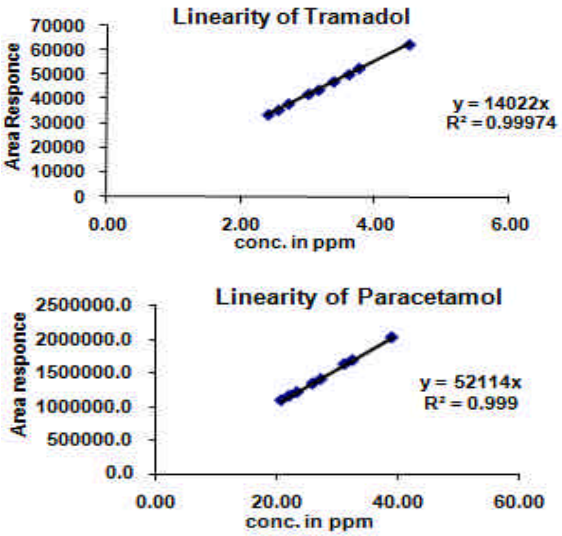

Fig.2. Linearity of Paracetamol and Tramadol

Robustness:
The robustness of the method is its ability to remain unaffected by small changes in parameters. Effect and slight change in flow rate (1.0 ± 0.2 mL/min), change in detection wavelength (228 ± 2 nm), and change in composition of mobile phase methanol: buffer (38:62, 40:60, 42:58) was checked. One factor at a time was changed to estimate the effect. Thus replicate injection (n = 3) of standard solution at same concentration levels were performed under small change of two chromatographic parameters (factors). Results presented in Table 3 indicate that the selected factors remain unaffected by small variation of the parameters. It was also found that there is no significant influence on retention time by change in such parameters and insignificant variability in retention time was observed.

RESULTS AND DISCUSSION
In this work the HPLC-UV method for analysis of TR and PR in a tablet formulation was developed and validated. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for PR and TR was obtained with a mobile phase consisting of methanol: buffer (40:60 v/v). Quantification of drugs was performed at 228 nm. Resolution of the two components was around 6.0 with clear baseline separation was obtained as shown in Figure 3. TR and PR were eluted around 2.1 and 3.9 min, respectively (Table 1).

Table 1. Results of Assay Experiment

Sr. No.	Drug	Labeled amount, mg	Retention time (min)	% Assay
1.	Paracetamol	325	3.9	99.23
2.	Tramadol	37.5	2.1	99.87

Method validation
The proposed HPLC method was validated in terms of linearity, precision (within-day and day-to-day), accuracy, system suitability test and robustness.

Accuracy:
The accuracy of the method was determined by calculating recoveries of PR and TR by the standard addition method. Known amounts of standard solutions of PR (20.8, 26.0, 31.2 and 39.0 µg/mL) and TR (2.4, 3.0, 3.6 and 4.5 µg/mL) were added to pre-quantified sample solutions. The amounts of PR and TR were estimated by applying these values to the regression equations of the calibration curves. The recovery obtained did not differ from the real value (± 2.0 %) (Fig. 3).

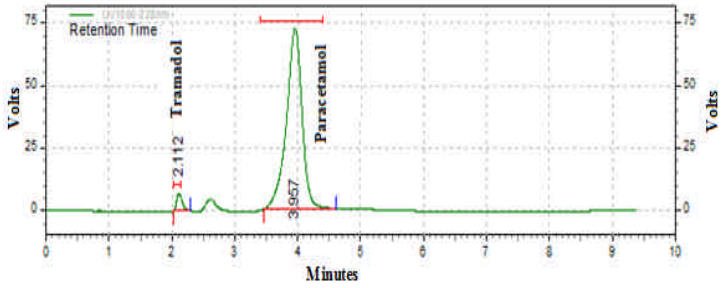

Fig.3. Accuracy of paracetamol and tramadol

Linearity:
Linearity correlation was obtained between peak area and concentrations of PR in range of 20.8 - 39.0 µg/mL and TR in range of 2.4 -4.5 µg/mL. The linearity of calibration curves was validated and correlation coefficient of regression was found near to 1 (0.999). The results showed that good correlation existed between the peak area and concentration of the analytes (Fig. 4).

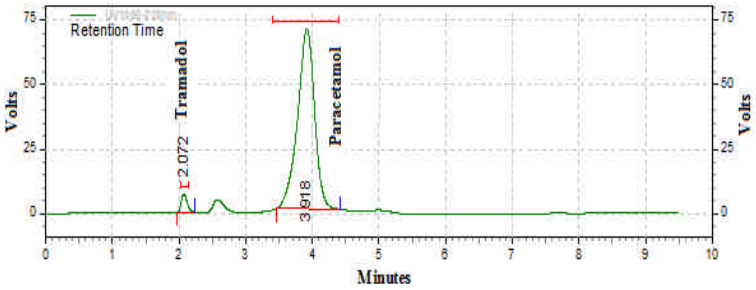

Fig.4. Linearity of paracetamol and tramadol

Method precision:
The instrumental precision was checked by repeatedly injecting (n = 6) solution of binary mixture containing PR (26.0 µg/mL) and TR (3.0 µg/mL) on same day (Fig. 5).

Intermediate precision:
The interday precisions of the proposed method was determined by estimating the corresponding responses (n = 6) times on different days for same concentrations of PR (26.0 µg/mL) and TR (3.0 µg/mL). The results presented in Table 2 are reported in terms of relative standard deviation (%RSD). The %RSD for twelve sample preparations (six of intraday and six of interday precision) was not more than 2.0 %.

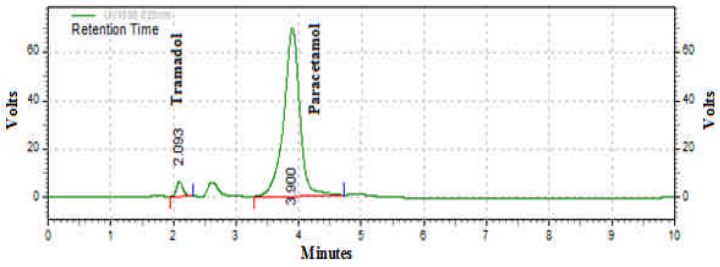

Fig.5. Intraday Precision of paracetamol and tramadol

System suitability test:

A solution was prepared at concentration of 3.0 µg/mL of TR and 26.0 µg/mL of PR was injected six times (n = 6), then the system suitability parameters were calculated from the chromatogram. The parameter such as retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results obtained from system suitability tests were in agreement with the USP requirements.

Table 2. Summary of validation parameters

Sr.no.	Parameter	Paracetamol	Tramadol
1	Linearity coefficient	0.99974	0.99986
2	Precision %RSD	0.16	0.19
3	Intermediate Precision % RSD	0.20	0.25
4	Solution stability % RSD 12 hrs	0.25	0.11
	%RSD 24 hrs	0.17	0.36
5	Retention times Minutes	3.9	2.1
6	Resolution	6.07	-

Robustness:

Robustness of the method was determined by making slightly changes in chromatographic conditions. Effect and slight change in method parameter like change in flow rate, change in detection wavelength, change in aqueous phase concentration, and change in organic phase concentration were applied. Thus replicate injections (n = 3) of standard solution at same concentration levels were performed under small changes of chromatographic parameter (factors). In all the studies the resolution between PR and TR peaks is greater than 5.5. The results presented in Table 3 indicate that the selected factors remain unaffected by small variation in these parameters.

Table 3. Robustness evaluation of the method

Sr. no.	Chromatographic change factor	Level		Paracetamol		Tramadol	
				Retention time (min)	(%) RSD	Retention time (min)	(%) RSD
1	Flow rate (ml/min)	0.8	-0.2	4.82	0.42	2.68	0.28
		1.0	0.0	3.90	0.10	2.19	0.14
		1.2	+0.2	3.23	0.43	1.79	0.41
2	Wavelength (nm)	226	-0.2	3.88	0.38	2.16	0.33
		228	0.0	3.89	0.13	2.18	0.16
		230	+0.2	3.60	0.36	2.14	0.32
3	Mobile phase (Methanol: Buffer)	38:62	-0.2	4.01	0.50	2.23	0.31
		40:60	0.0	3.89	0.19	2.19	0.28
		42:58	+0.2	3.74	0.32	2.09	0.81

CONCLUSION

The developed HPLC method for simultaneous determination of PR and TR in a drug sample from a pharmaceutical quality control offers the critical advantage of complete separation within 5.0 min. The time required for separation of these two drugs is significantly lower than previous reported HPLC methods where TR get eluted after elution of PR but in our method TR get separated before separation of PR between 2.0 to 2.1 min. The analytical method is simple, robust and adequately validated and convenient for separation of these two compounds that can be used for the assay of their respective dosage form. The validation parameters were also found in acceptable of FDA and ICH guidelines.

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