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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF PARACETAMOL AND TAPENTADOL HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Paracetamol and Tapentadol HCl in pharmaceutical dosage form. The column used was Symmetry C18 (4.6 x 150mm, 5µm) in isocratic mode, with mobile phase containing phosphate buffer-acetonitrile (50:50 v/v) adjusted to pH 3.6 with ortho phosphoric acid. The flow rate was 0.8 mL/ min and effluents were monitored at 243 nm. The retention times of Paracetamol and Tapentadol HCl were found to be 2.206 min and 3.807 min, respectively. The linearity for Paracetamol and Tapentadol HCl were in the range of 100-200 mcg/mL and 15-30 mcg/mL respectively. The recoveries of Paracetamol and Tapentadol Hcl were found to be 98.5% and 98.5%, respectively. The proposed method was validated and successfully applied to the estimation of Paracetamol and Tapentadol Hcl in combined tablet dosage forms.

Keywords: Validation, RP-HPLC, Paracetamol, Tapentadol HCl.

INTRODUCTION

Paracetamol is classified as anti pyretic and analgesic. It is commonly used for the relief of headache and other pains, and is a major ingredient in numerous cold and flu remedies. Its chemically known as N- (4- hydroxyphenyl) ethanamide N- (4-hydroxyphenyl) acetamide. Tapentadol HCl is a centrally acting analgesic with a dual mode of action as an agonist of the µ-opioid receptor and as a norepinephrine reuptake inhibitor. It is also an agonist of the σ_2 receptor, though the function of this orphan receptor remains controversial. While its analgesic actions have been compared to tramadol and oxycodone, its general potency is somewhere between tramadol and morphine in effectiveness. It has opioid and nonopioid activity in a single compound. It is chemically 3-[(1R, 2R)-3-(dimethylamino)-1-ethyl-2-methylpropyl] phenol hydrochloride . Literature survey revealed that few analytical methods have been reported for the determination of Paracetamol and Tapentadol HCl in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography either in single or in combined forms[1-7]**.** Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the simultaneous determination of Paracetamol and Tapentadol HCl in bulk and in tablet dosage form.

EXPERIMENTAL

Chemicals and reagents: Paracetamol and Tapentadol HCl were obtained from M/s Mercury Laboratories Ltd., (Vadodara, India). Methanol and acetonitrile (HPLC grade) were purchase from (E.

Merck, Mumbai, India). Potassium dihydrogen phosphate, phosphoric acid, and NaOH (S. D. Fine Chemicals, Mumbai, India) used were of analytical grade. Double distilled water was used throughout the study.

Equipment: The HPLC (Waters) instrument was equipped with a model series Water's 2695 binary gradient pump, an inbuilt auto sampler, a column oven and 2487 dual wavelength absorbance detector (DAD). Data acquisition was performed on Empower 2 software.

Chromatographic condition: The HPLC separation and quantitation were achieved on a Symmetry C18 (4.6 x 150mm, 5µm, Make: Kromosil) column. The mobile phase was prepared by mixing 10 mM KH_2PO_4 (pH 3.6) – acetonitrile (50:50, v/v) that run isocratically at a flow rate 0.8 mL/min. All determinations were performed at ambient temperature. The injected volume was 20 µL. The detector was set at λ 243 nm.

Preparation of standard: Paracetamol and Tapentadol were weighed (100mg each) and transferred to two separate 100 ml volumetric flasks and dissolved in mobile phase, which gives 1000 µg/mL of Paracetamol and Tapentadol. This solution was used as stock solution. Further working standards were prepared from the above stock solution.

Determination of Paracetamol and Tapentadol HCl in their combined dosage forms: The content of twenty tablets were weighed. Powder equivalent to 612 mg was accurately weighed and transferred to a 100 ml volumetric flask and 60 ml of mobile phase was added to the same and flask was sonicated for 5 min. The flask was shaken, and the volume was diluted to the mark with the same mixture. The above solution was filtered using Whatman filter paper No.1, Further pipette out 0.46ml of above stock solution into a 10ml of volumetric flask and dilute up to the mark with diluent to obtain 150 µg/ mL of Paracetamol and 27µg/mL of Tapentadol. The solution was injected at above chromatographic conditions and peak areas were measured.

Linearity: Appropriate aliquots of Paracetamol and Tapentadol stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 100, 125, 150, 175, 200 µg/mL of Paracetamol and 15, 19, 23, 27, 30 µg/mL of Tapentadol. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for Paracetamol and Tapentadol HCl.

Accuracy: The accuracy of the method was determined by calculating recoveries of Paracetamol and Tapentadol by method of standard additions. Known amount of Paracetamol and Tapentadol were added to a pre quantified sample solution, and the amount of Paracetamol and Tapentadol were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Precision: Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard concentrations of 150µg/ml and 23µg/ml for Paracetamol and Tapentadol and results are reported in terms of relative standard deviation (RSD).

Specificity: Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution. No interference was detected at the retention times of both Paracetamol and Tapentadol HCl in sample solution.

Robustness: To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in flow rate and ± 10% change in mobile phase

RESULTS AND DISCUSSION

The present study was aimed at developing a simple, economical, precise and accurate HPLC method for the analysis of Paracetamol and Tapentadol HCl in bulk drug and in pharmaceutical dosage form. In order to achieve simultaneous elution of the two components, initial trails were performed with the objective to select adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wavelength, optimum pH, different columns and concentration of the standard solutions were carefully studied. Several solvents were tested by using different proportions, such as methanol-water (80:20 v/v), acetonitrile-water (80:20 v/v), methanol-0.05M phosphate buffer (80:20 v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid), methanol-acetonitrile-0.05M

phosphate buffer (80:10:10 v/v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid) and methanol-acetonitrile-0.05M phosphate buffer (60:20:20 v/v/v, pH 3.5-6.5 adjusted with ortho-phosphoric acid). Finally, a mixture of Phosphate buffer (pH 3.6 adjusted with ortho phosphoric acid) and acetonitrile in a proportion of 50:50 v/v was selected as the optimum mobile phase and a flow rate of 0.8 mL/min. Under these conditions, the analyte peaks were well resolved and were free from tailing. The tailing factor was <1.5 for both the analytes. The retention time obtained for paracetamol was 2.206 min and for Tapentadol HCl was 3.807 min. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of Paracetamol and Tapentadol were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.999) was observed for Paracetamol and (r^2=0.999) was observed for Tapentadol. The detection limit for Paracetamol and Tapentadol HCl were 0.07µg/mL and 0.06µg/mL, respectively. The quantitation limit for Paracetamol and Tapentadol HCl were 0.23µg/mL and 0.21µg/ml, respectively.

The proposed method was applied to the simultaneous estimation of Paracetamol and Tapentadol HCl in tablets. The assay results show that the proposed method was selective for the simultaneous determination of Paracetamol and Tapentadol HCl without interference from the excipients used in the tablet dosage form. The values were shown in Table 2. The assay results and low %RSD values indicated that the developed method can be used for routine analysis of Paracetamol and Tapentadol HCl in pharmaceutical dosage forms.

Table 1: Validation parameters and data for proposed method

Validation parameter	***Paracetamol***	***Tapentadol***
Linearity	100-200 µg/mL	15-30 µg/mL
Regression coefficient (r^2)	0.999	0.999
Detection limit (µg/mL)	0.007	0.06
Quantitation limit (µg/mL)	0.23	0.21
Accuracy (% recovery)	98.5	98.5
Precision		
Repeatability of injection (%RSD)	0.17	0.42
Intermediate precision (%RSD)	0.08	0.08
Assay value (%)	100.2	99.7
System suitability parameter		
Tailing factor	1.2	1.1
Theoretical plate	2005	2795
Resolution	6.3	

** Replicates of three concentration levels (in three determinations); ** Ten repetitive injections of same homogeneous sample*

Table 2: Estimation of amount present in tablet dosage form

Brand Name	Tablet Formulation	Label Claim per Tablet (mg)	% Label claim estimated (Mean± SD) N=3	% Drug estimated
Zyntap P	Paracetamol	325	325.65	100.2
	Tapentadol HCl	50	49.85	99.7

Table 3: The accuracy results for Paracetamol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2220290	4.8	4.70	98.1%	98.5%
100%	4680084	10.0	9.92	99.2%	
150%	6875885	14.86	14.5	98.1%	

Table 4: The accuracy results for Tapentadol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	543055	4.79	4.69	98.0%	98.5%
100%	1151367	10.0	9.95	99.5%	
150%	1698958	14.98	14.6	98.0%	

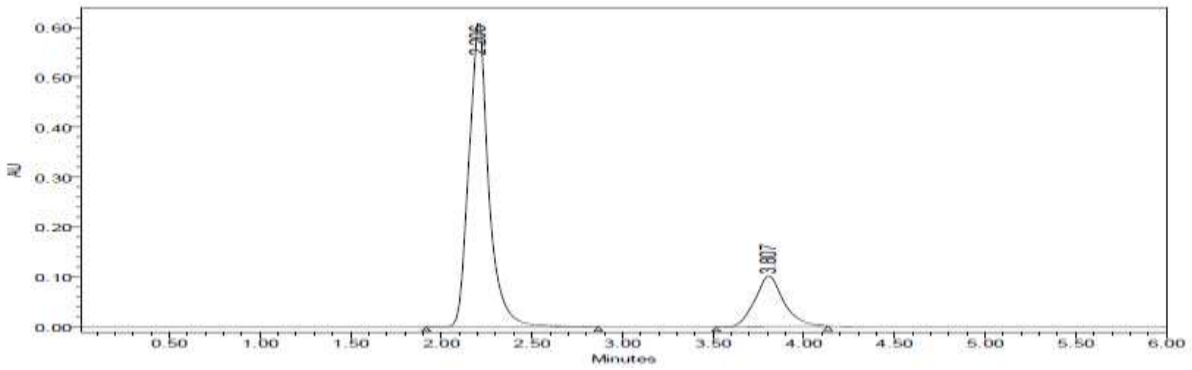

Figure 1: HPLC chromatogram of Paracetamol and Tapentadol HCl in optimized chromatographic conditions

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