IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 4, Issue 6 (June 2014), PP. 33-38

Simultaneous Estimation of Paracetamol and Tapentadol in Bulk and Pharmaceutical Formulation by Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

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Abstract: *Paracetamol and Tapentadol are both analgesic drugs. As there is no UV or HPLC method for the simultaneous estimation of Paracetamol and Tapentadol, a need was felt to develop the method for the analysis of both drugs simultaneously. This work concerns with the development and validation of a simple, specific and cost effective RP-HPLC method for simultaneous estimation of Paracetamol and Tapentadol in bulk and the developed method was applied to the pharmaceutical dosage form ie., Tapcynta. Chromatography was carried on Thermohypersil BDS C18 column with mobile phase comprising of Dihydrogen potassium phosphate buffer and Acetonitrile in the ratio of 50:50 v/v. The flow rate was adjusted to 0.9 ml/min with PDA detection at 218.6 nm. The retention times of Paracetamol and Tapentadol were found to be 4.8 min, 8.0 min respectively and other replicate standard system suitability parameters are within the limit and uniform. The different analytical parameters such as accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response was linear in the range of 88044x + 19214 mg/ml, 49855x + 36868 mg/ml for Paracetamol and Tapentadol respectively. The proposed method was successfully applied for the reliable quantification of active pharmaceuticals present in the commercial formulations.*
Keywords: *Paracetamol, Tapentadol, Simultaneous, Estimation, HPLC*

I. INTRODUCTION:

Tapentadol HCl (Fig.1) 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 antagonist of the σ2 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. Paracetamol (Fig. 2) 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. It is chemically known as N- (4- hydroxyphenyl) ethanamide N- (4- hydroxyphenyl) acetamide.

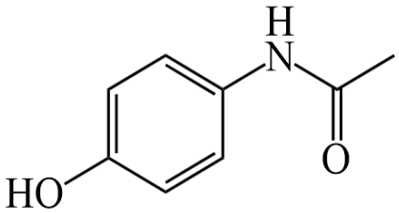

Fig-1

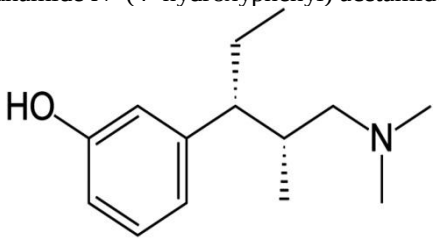

Fig-2

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.

1. Materials and Methods:

2.1. Materials:

2.1.1. Chemicals and Reagents:

Working standards of pharmaceutical grade Paracetemol and Tapentadol were obtained as generous gifts from Dr.Reddy's laboratories (Hyderabad, AP, India) used as such without further purification. The pharmaceutical dosage form used in the study was Tapcynta.

Acetonitrile (HPLC grade), Potassium dihydrogen phosphate (AR grade) purchased from Merck specialities Pvt.ltd (Mumbai, India) and double distilled water used for analysis.

1.1.2. Instrumentation and chromatographic condition:

Chromatography was carried out on thermohypersil BDS C18 column (250x4.6x5) with mobile phase comprising of Potassium dihydrogen phosphate buffer and Acetonitrile in the ratio of 50:50. The flow rate was adjusted to 0.9 ml / min with PDA detection at 218.6 nm.

1.1.3. Preparation of standard solution:-

Standard stock solutions of pure drugs were prepared separately by dissolving 5 mg of Tapentadol in 25 ml water and 32.5 mg of Paracetamol in 10ml water to get concentrations 0.2 mg/ml and 1.3 mg /ml respectively.

1.1.4. Preparation of sample solution:

20 tablets were weighed accurately, powdered and equivalent weight was calculated. The equivalent weight of two tablets were taken and dissolved in 100 ml of water to get the concentration 1 mg/ml of Tapentadol and 6.5 mg /ml of Paracetamol. From stock solution 0.5 ml was taken and diluted to 25 ml with water to get concentrations 0.2 mg/ml Tapentadol and 1.3 mg/ml of Paracetamol.

1.2. Validation:

The developed method was validated with different analytical parameters such as accuracy, precision, linearity, limit of detection, limit of quantification and robustness according to the international conference on harmonization (ICH) Q2B guidelines.

2.2.1. Precision:

Precision of these methods was checked by analyzing the samples at three different time intervals of the same day (intraday precision (table-2)) as well as on different days (interday precision). Robustness for HPLC method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 0.9 mL/min to 0.7 mL/min and 1.1 mL/min while ratio of the mobile phase was changed by ± 1%.

Table-2: Validation parameters:

Validation Parameter	**Tapentadol**	**Paracetamol**
System precision	0.1%	0.1%
Tailing factor	1.2	1.3
Theoretical plate count	18870	8450
Linearity	100-300 µg/ml,	650-1950 µg/ml
Regression equation	y = 49855x + 36868	y = 88044x + 19214
Regression coefficient	0.999	1
Detection limit (µg/mL)	0.45µg/ml	0.83 µg/ml
Quantitation limit (µg/mL)	1.37µg/ml	2.52 µg/ml

Accuracy (% recovery)	100%	100%
Precision		
Intra-day Precision		
Assay value	100%	99%
%RSD	0.14	0.25
Inter-day Precision		
Assay value	100%	99%
%RSD	0.3	0.3

1.2.2. Recovery studies:

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100, 150% levels (table-3) . From the total amount of drug found percentage recovery was calculated.

Table-3: Recovery studies of Tapentadol

%Concentration	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	2579374	0.1	0.099	99%	
100%	5071934	0.2	0.20	100 %	100%
150%	7531320	0.3	0.299	99.8%	

Table-3: Recovery studies of Paracetamol

%Concentration	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	443595	0.65	0.645	100%	
100%	8845898	1.3	1.310	100 %	100%
150%	13258959	1.95	1.948	100%	

1.2.3. Linearity:

LOD and LOQ:

Limit of Detection (LOD) and Limit of quantification (LOQ) were calculated by using the values of slopes and intercepts of the calibration curves for both the drugs. LOD and LOQ values for Tapentadol were found to be 0.45 µg/ml and 1.37 µg/ml and for Paracetamol 0.83 µg/ml and 2.52 µg/ml respectively.

1.2.4. Robustness:

Method robustness was determined by the small changes in chromatographic conditions like as 0.2ml flow rate and ±5ºc temperature and inject the sample observe the result there were no marked changes compare to other analysis. Results of the Robustness were shown in table-3.

Table-4: Robustness study

Parameters	**Changes**	**Retention Time**
Tapentadol		
Flow rate (ml/min)	0.7	7.95
	1.1	4.73
Temperature	40°C	5.96
	50°C	6.06
Paracetamol		
Flow rate (ml/min)	0.7	4.25
	1.1	2.50
Temperature	40°C	3.15
	50°C	3.13

II. RESULTS AND DISCUSSION:

Retention times of Tapentadol and Paracetamol were found to be 5.9 and 3.1 respectively (as shown in Fig. 3). The detector response was linear in the range of 100-300 µg/ml, 650-1950 µg/ml for Tapentadol and Paracetamol respectively. In the linearity study the regression equation and coefficient of correlation for Tapentadol and Paracetamol were found to be ($y = 49855x + 36868$, $R^2 = 1$), ($y = 88044x + 19214$, $R^2 = 0.999$) respectively. Commercial formulations containing Tapentadol and Paracetamol were analyzed by the proposed method. A typical chromatogram of marketed formulation is shown in fig. no.3. Six replicate analysis of formulation were carried out and the mean assay values were found close to 100 %. The tailing factors were <2.0 for both the peaks. The elution order was Paracetamol (RT = 3.1 min) and Tapentadol (RT = 5.9 min), at a flow rate of 0.9 mL/min. The chromatogram was recorded at 218.6 nm. System suitability was established by injecting standard solution and results are shown in table no.1.The accuracy of the proposed method was determined by recovery studies. It was confirmed from results that the method is highly accurate (table no.2 and 3). Precision (table no.1) was calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for intraday and interday precision for Tapentadol were 0.14 % and 0.3 % and that for Paracetamol were 0.25 % and 0.31 % respectively which are well within the acceptable limit of 2 %. For robustness studies in all deliberately varied conditions, the RSD of contents of Tapentadol and Paracetamol were found to be well within the acceptable limit of 2%.

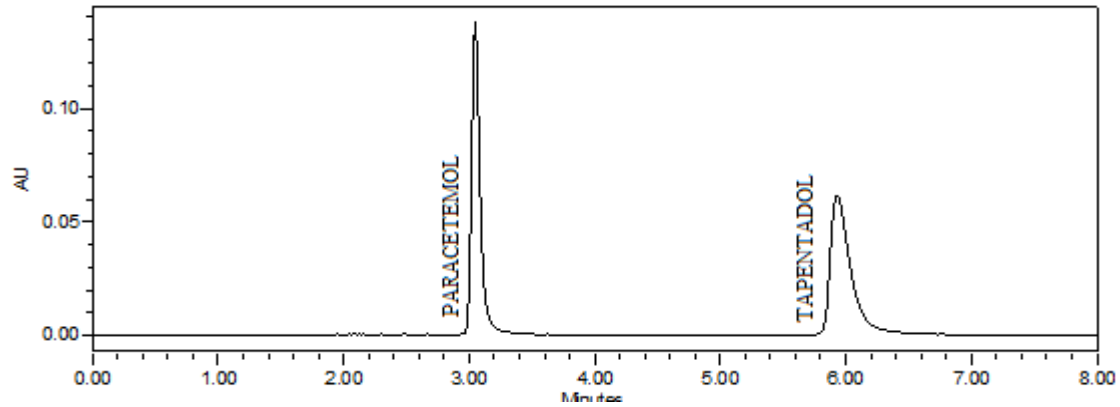

Fig. 3

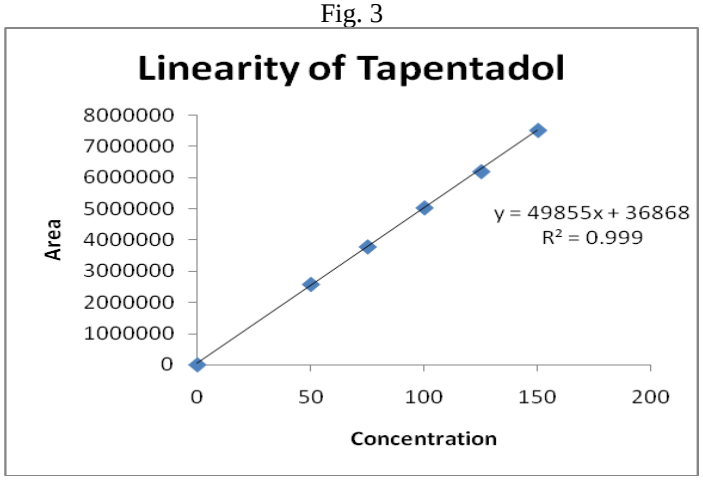

Fig. 4

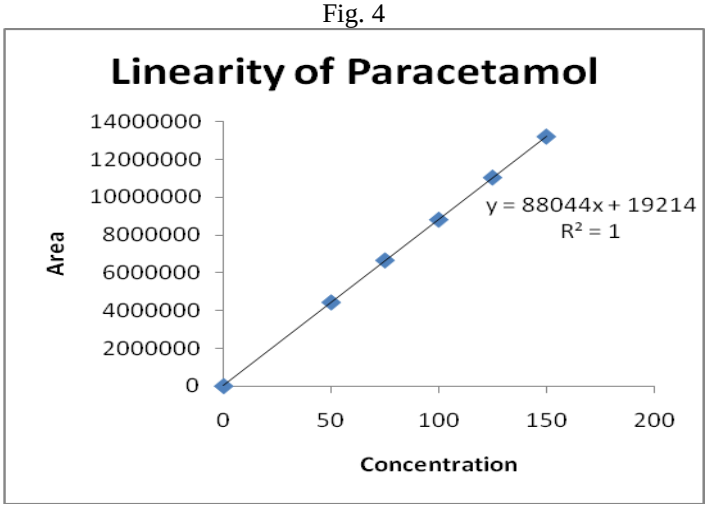

Fig. 5

III. CONCLUSION:

The new HPLC method developed and validated for simultaneous estimation of Tapentadol and Paracetamol pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, precise, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

ACKNOWLEDGEMENT:

Authors thank full to Rainbow Pharma training lab, Kukatpally, Hyderabad, for providing instruments and analytical support.

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