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Development and Validation of Stability Indicating RP-LC Method for Simultaneous Estimation of Tapentadol and Paracetamol in Bulk and Its Pharmaceutical Formulations.

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for simultaneous estimation of Tapentadol and Paracetamol in tablet formulations. The separation was achieved by using C-18 column (Inertsil ODS 3v, 150 x 4.6mm i.d.) in mobile phase pH 2.5 Phosphate Buffer and Acetonitrile in the ratio of 800:200 v/v. The flow rate was 1.5 mL.min-1 and the separated drugs were detected using UV detector at the wavelength of 215 nm. The retention time of Tapentadol Hydrochloride, and Paracetamol, was noted to be 4.65 and 2.39, respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid Chromatography; Tapentadol, Paracetamol, Combined dosage forms; Simultaneous estimation, Validation

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INTRODUCTION

Tapentadol [5, 10-11] is a novel centrally acting analgesic that was approved for use by the Food and Drug Administration in November 2008. It has structural similarities to tramadol. The drug has a unique mode of action in that it functions as an agonist at the μ-opioid receptor, and as a norepinephrine reuptake inhibitor. This dual mode of action provides analgesia at similar levels of more potent narcotic analgesics such as hydrocodone, oxycodone and morphine, but with a more tolerable side effect profile. The chemical name is 3-[(1*R*,2*R*)-3-(dimethylamino)-l-ethyl-2-methylpropyl]phenol monohydrochloride. The structural formula is: The molecular weight of tapentadol HC1 is 257.80, and the molecular formula is $C_{14}H_{23}NO{\cdot}HCl$. The n-octanol:water partition coefficient log P value is 2.87. The pKa values are 9.34 and 10.45

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drugs. Paracetamol is available indifferent dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories. Dosage forms of paracetamol and its combinations with other drugs have been listed in various pharmacopoeias [1-3, 10, 11]. Paracetamol (p-hydroxy acetanilide) is a compound with analgesic and antipyretic properties. It is much safer than aspirin in terms of gastric irritation, ulceration and bleeding.

Figure 1: The structures of Paracetamol and Tapentadol.

A. Tapentadol **B. Paracetamol**

Literature survey revealed a few HPLC methods for the estimation of Paracetamol and Tapentadol [5-9]. No HPLC method for simultaneous estimation of these two drugs has been reported till date. In the present study, an attempt has been made to develop a method for the simultaneous estimation of two drugs- Paracetamol (I) and Tapentadol (II). It can also be applied for routine analysis of either one or of any combinations of in these drugs dosage forms.

EXPERIMENTAL

Chemicals and Reagents

Milli-Q Water, Methanol (HPLC Grade), Orthophosphoric acid (GR Grade), disodium hydrogen phosphate (GR Grade) were obtained from Merck Ltd., Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation and Chromatographic Conditions

Instrumentation

The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Inertsil ODS 3v ((Make: GL Sciencies); 150 mmx4.6 mm I.D; particle size 5 μm)) was used. The output of signal was monitored and integrated using waters Empower 2 software.

Buffer preparation

Dissolve 3.2g of Disodium hydrogen Phosphate in 1000mL of Milli-Q Water, adjust pH to 2.5 with dilute ortho phosphoric acid and Filter the solution through 0.45μm membrane filter.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 800:200 v/v respectively.

Diluent preparation

Mobile Phase is used as diluent.

Standard preparation:

Accurately weigh and transfer about 130mg of Paracetamol and 20.0mg of Tapentadol into a 50 mL volumetric flask, add 30 mL of diluent and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluent.

Sample preparation

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 325mg of Paracetamol and 50mg of Tapentadol into a 100 mL volumetric flask, add 30 mL of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 10.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent.

Chromatographic conditions

An Inertsil ODS 3V ((Make: GL Sciencies; 150 mmx4.6 mm I.D; particle size 5µm)) Column was used for analysis at 40°C column temperature. The mobile phase was pumped through the column at a flow rate of 1.5mL/min. The sample injection volume was 20 µL. The photodiode array detector was set to a wavelength of 215nm for the detection and Chromatographic runtime was 6 minutes.

RESULTS AND DISCUSSION

Method development [1-5]

Spectroscopic analysis of compounds showed that (I) and (II) have maximum UV absorbance (λmax) at 215 nm(For Tapentadol), 242 nm(For Paracetamol) respectively. Therefore, the chromatographic detection was performed at 215nm using a Photo diode array detector as paracetamol also exhibits good response at Tapentadol λmax . To develop a suitable and robust LC method for the determination of Tapentadol and Paracetamol, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Inertsil ODS 3v ((Make: GL Sciencies; 150 mmx4.6 mm I.D; particle size 5 µm)) with the following mobile phase. Accurately transfer about pH 2.5 Phosphate buffer. Filter the solution through 0.45µm membrane filter. Prepare a filtered and degassed mixture of Buffer and acetonitrile in the ratio of 600:400 v/v respectively. It was observed that when a combination of two drugs was injected, Paracetamol and Tapentadol together gave a single split M shape peak.

For next trial the mobile phase composition was changed slightly. The mobile phase composition was Buffer and acetonitrile in the ratio of 700:300 v/v. above trail the peak shape was little broad but the peaks are separated. Again the mobile phase composition changed slightly to Buffer and acetonitrile in the ratio of 800:200 v/v respectively as eluent at flow rate 1.5 mL/min. UV detection was performed at 215nm. The retention time of Paracetamol is 2.39 minutes and Tapentadol is about 4.65 (refer Fig-2.) and the peak shape for these two was good.

Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. Different experiments were performed to optimize the mobile phase and adequate separation of drugs achieved. The optimized mobile phase was determined as a mixture of Buffer and acetonitrile (800:200) at a flow rate of 1.5 mL.min-1. Under these conditions (I) and (II) were eluted at 2.39 and 4.65, minutes respectively with a run time of 6 min.

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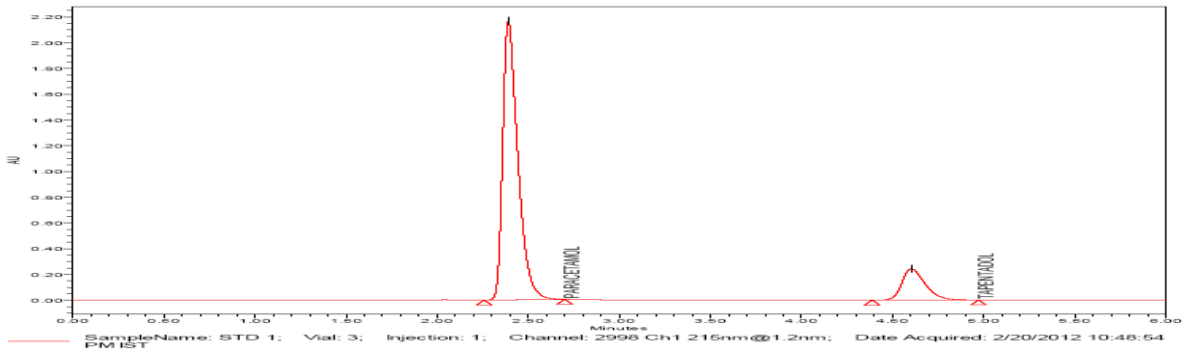
The chromatogram of Paracetamol and Tapentadol standard using the proposed method is shown in (Fig-2.) System suitability results of the method are presented in Table-1.

A typical chromatogram for simultaneous estimation of (I) and (II) obtained by using the aforementioned mobile phase from 20 µL of the assay preparation is illustrated in Fig. 2.

Figure 2: A typical HPLC Chromatogram showing the peak of Paracetamol and Tapentadol



Method validation [13-15]

The developed RP-LC method extensively validated for assay of Paracetamol (I), and Tapentadol (II) using the following Parameters.

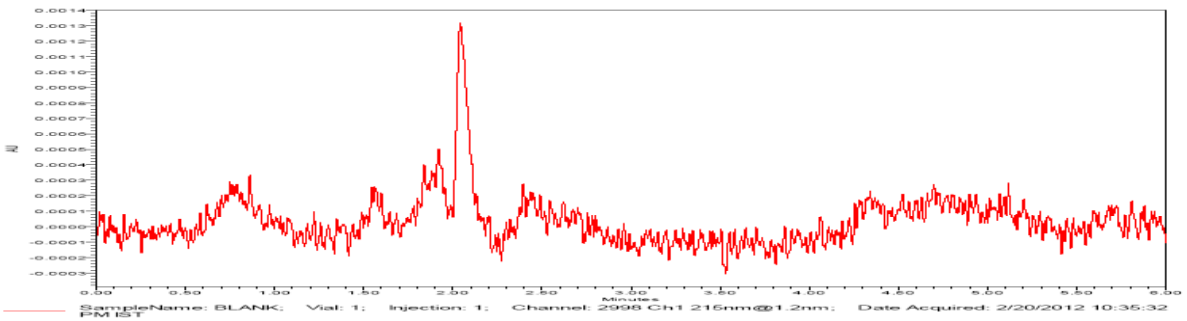
Specificity:

Blank and Placebo interference:

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution (Fig. no.-3) showed no peaks at the retention time of Paracetamol and Tapentadol peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Paracetamol and Tapentadol in Paracetamol and Tapentadol tablets. Similarly Chromatogram of Placebo solution (Fig. no.-4) showed no peaks at the retention time of Paracetamol and Tapentadol peak. This indicates that the Placebo used in sample preparation do not interfere in estimation of Paracetamol and Tapentadol in Paracetamol and Tapentadol tablets.

The chromatogram of Paracetamol and Tapentadol Blank using the proposed method is shown in **Fig- 3**.

Figure3: A typical HPLC Chromatogram showing the no interference of diluent for Paracetamol and Tapentadol



The chromatogram of Paracetamol and Tapentadol Placebo using the proposed method is shown in **Fig-4**.

ISSN: 0975-8585

Figure 4: A typical HPLC Chromatogram showing the no interference of placebo for Paracetamol and Tapentadol

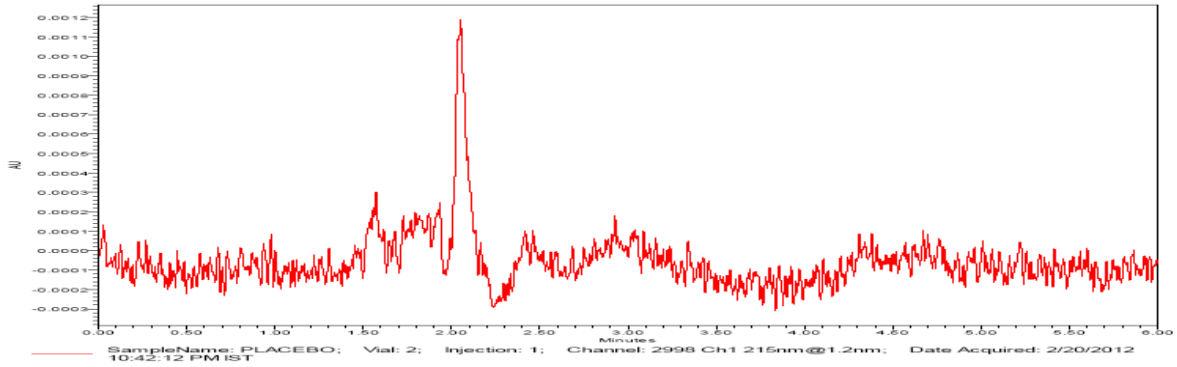


Table 1: System suitability parameters for Paracetamol and Tapentadol by proposed method

Name of the Compound	Retention Time	Theoretical plate	Tailing factor
Paracetamol	2.51	1.25	2025
Tapentadol	4.27	1.43	4353

Forced Degradation

Control Sample

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 325.mg of Paracetamol and 50mg of Tapentadol into a 50 mL volumetric flask, add 30 mL of diluent, and sonicate for 50 minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer105.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent.

Acid Degradation Sample

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 325.mg of Paracetamol and 50mg of Tapentadol into a 100 mL volumetric flask, add 30 mL of diluent, and sonicate for 15minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 10.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-5A)

Base Degradation Sample

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 325.mg of Paracetamol and 50mg of Tapentadol into a 100 mL volumetric flask, add 30 mL of diluent, and sonicate for 15minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N NaOH, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 10.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-5B)

Peroxide Degradation Sample

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 325.mg of Paracetamol and 50mg of Tapentadol into a 100 mL volumetric flask, add 30 mL of diluent, and sonicate for 15minutes with intermittent shaking at controlled temperature. Then add 5mL of Hydrogen Peroxide, refluxed for 30min at 60°C, then cooled to room temperature, and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 10.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-5C)

ISSN: 0975-8585

Thermal Degradation Sample

Powder collected from 20 tablets are exposed to heat at 105°C for about 5days. Accurately weigh and transfer equivalent to 325.mg of Paracetamol and 50mg of Tapentadol into a 50 mL volumetric flask, add 30 mL of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 10.0 mL of the above solution into a 25 mL volumetric flask and dilute to volume with diluent. Refer (Fig. no.-5D)

Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

Figure 5A: A typical HPLC Chromatogram showing the degradation profile of Tapentadol and Paracetamol in Acidic hydrolysis by proposed method

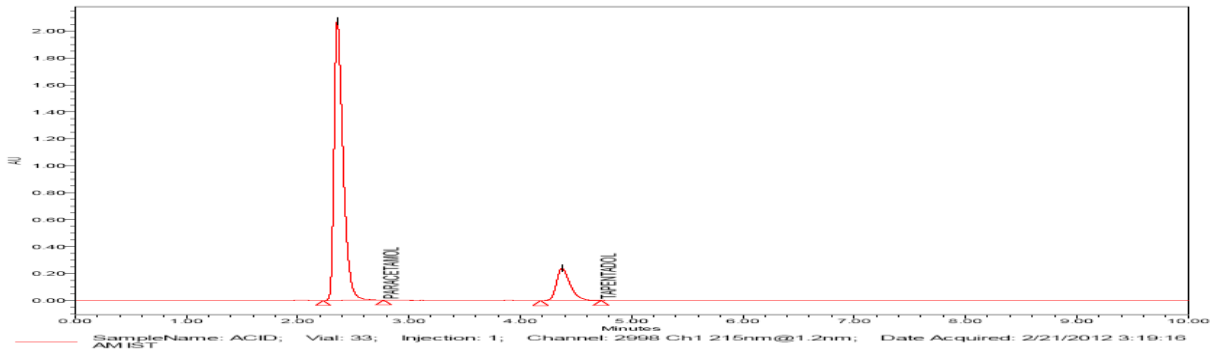


Figure 5B: A typical HPLC Chromatogram showing the degradation profile of Tapentadol and Paracetamol in Basic hydrolysis by proposed method

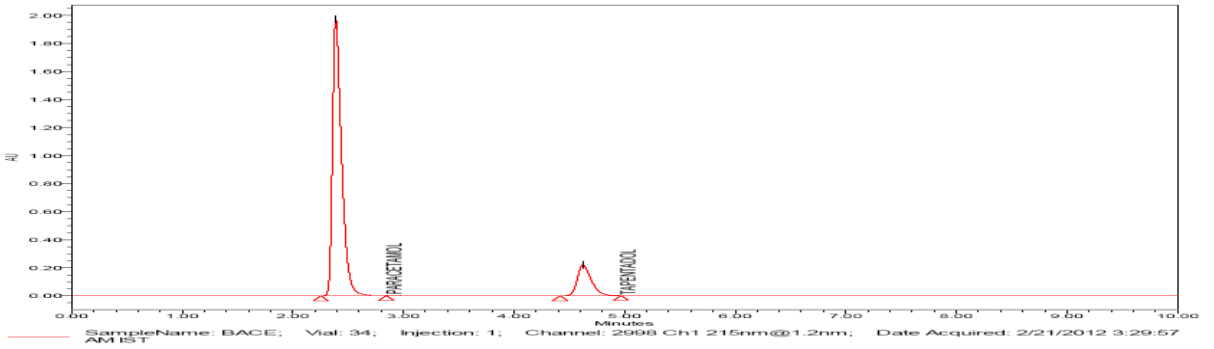
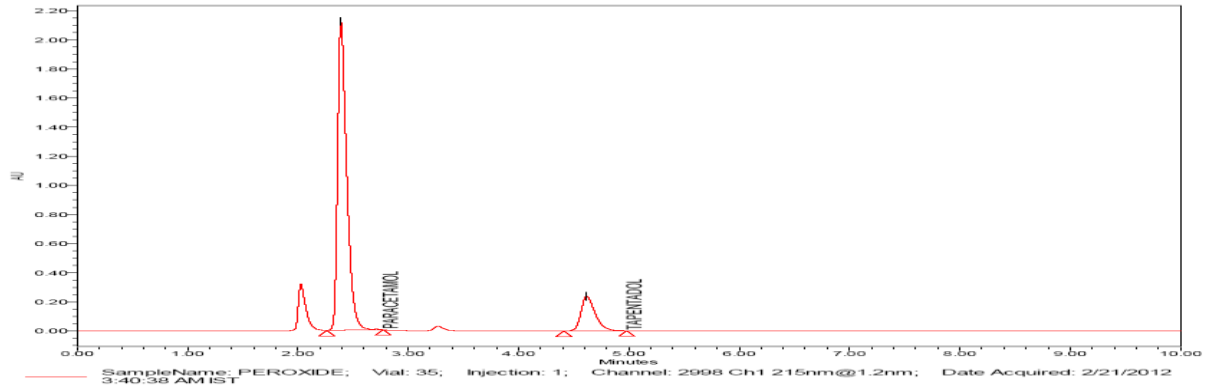
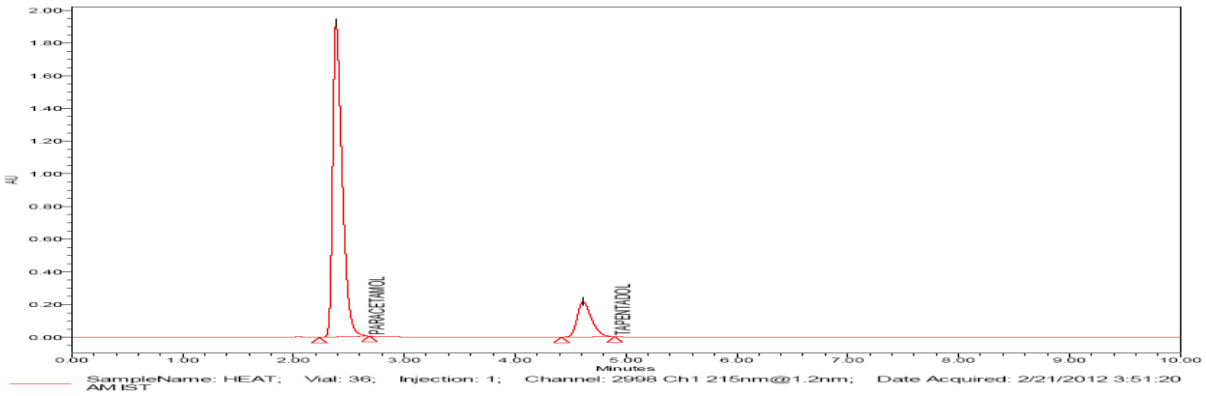


Figure 5C: A typical HPLC Chromatogram showing the degradation profile of Tapentadol and Paracetamol in Peroxide hydrolysis by proposed method.



ISSN: 0975-8585

Figure 5D: A typical HPLC Chromatogram showing the degradation profile of Tapentadol and Paracetamol in Thermal degradation by proposed method.



Precision

In the study of the instrumental system precision where, a RSD of 0.41% was obtained for the standard area of Paracetamol and 0.21 for Tapentadol obtained corresponding to the first day, Similarly being 0.2% for Paracetamol and 0.3% for Tapentadol for the second day, respectively. The method precision study for six sample preparations in marketed samples showed a RSD of 0.5% and with the assay range of 98.6-99.9 for Paaracetamol. Similarly The method precision study for six sample preparations in marketed samples showed a RSD of 0.7% and with the assay range of 99.2-100.7 for Tapentadol.

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 0.5 % (For Standard of Paracetamol) And 0.4% (For Standard of Tapentadol). The same study was carried out for different analysts (*n* = 6 number of samples per analyst) obtaining a RSD of 0.3 % (Intermediate Precision) and the assay range of 98.8-100.1 for Paracetamol. Similarly, obtaining a RSD of 0.3 % (Intermediate Precision) and the assay range of 99.5-100.4 for Tapentadol. The Overall %RSD for n=12 is 0.5. for Paracetamol and 0.5 for Tapentadol. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Table 2: Method Precision (Inter and Intraday) studies for Paracetamol and Tapentadol HCl by proposed method

Summary showing Method Precision by Proposed Method			
For Tapentadol		For Paracetamol	
Method Precision (Inter &Intra Day)		Method Precision (Inter &Intra Day)	
100.1	100.20	99.4	99.9
100.7	100.4	99.7	100.1
99.4	99.6	98.7	98.8
99.3	99.7	98.7	99.0
99.2	99.5	98.6	99.0
100.5	99.6	99.2	99.2
Overall Avg.	99.9		99.2
Overage Std Dev.	0.51		0.49
Over all %RSD	0.50		0.50

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Tapentadol and Paracetamol, analyzed as per the proposed method. The percentage recoveries with found in the range of 99.3 to 100.9 with an overall %RSD of 0.5 for Paracetamol and The percentage recoveries with found in the range of 100.1 to 100.6 with an overall %RSD of 0.2 for Tapentadol. From the data obtained which given in Table-3A and Table-3B the method was found to be accurate.

ISSN: 0975-8585

Table 3A: Recovery studies for Paracetamol by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	100.6-100.9	0.1	0.5
100	99.3-100.0	0.4	
150	99.7-99.8	0.1	

Table 3B: Recovery studies for Tapentadol by proposed method

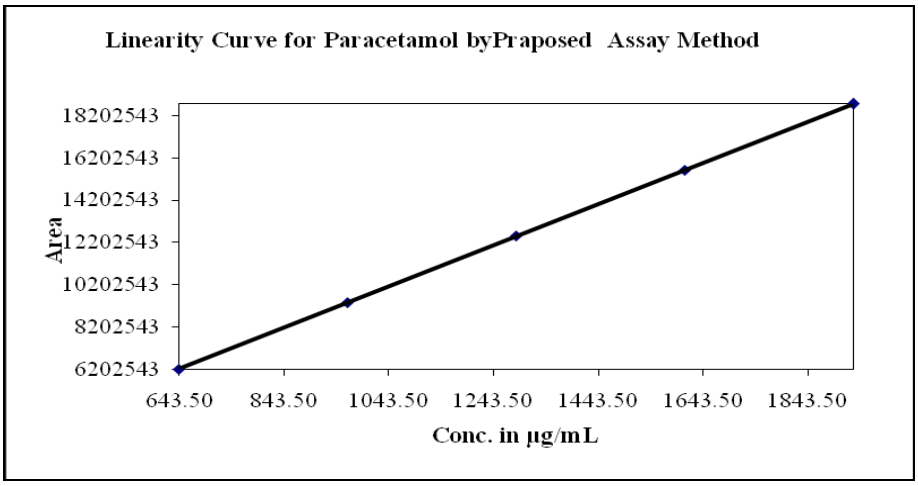
% Level	Recovery Range	% RSD at each level	Over all %RSD
50	100.2-100.5	0.1	0.2
100	100.1-100.6	0.3	
150	100.3-100.5	0.1	

Linearity of detector response

Table 4A: Linearity studies for Paracetamol by proposed method

Linearity Study for Paracetamol		
% Level	**Conc. µg/mL**	**Area**
50	643.50	6202543
75	965.25	9373723
100	1287.00	12522835
125	1608.75	15626825
150	1930.50	18744544
Slope		9740.0
Intercept		-40748.0
% Y-Intercept		-418.4
Residual Sum of Squares		25001.0
CC(r)		1.0000
RSQ(r2)		1.0000
LLD		8.47
LLQ		25.67

Figure 6A: Calibration curve for Paracetamol



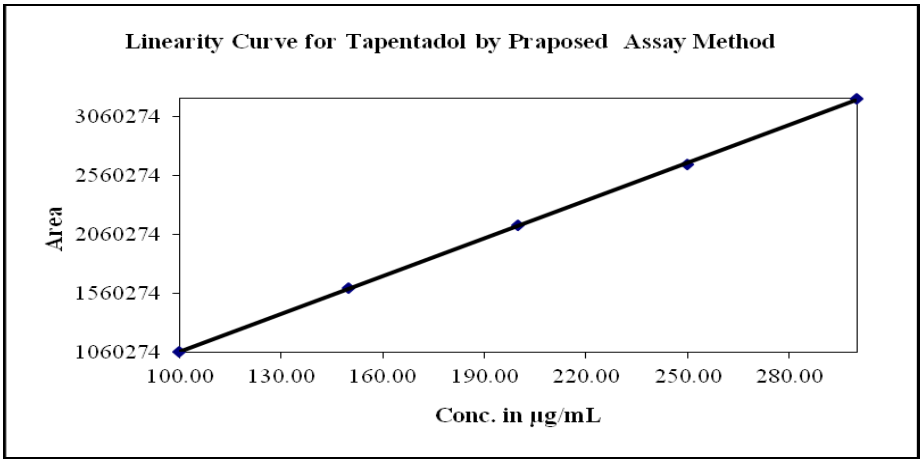
The standard curve was obtained in the concentration range of 643.50-1930.50µg/ml for Paracetamol and 100-300µg/mL for Tapentadol. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure-6A(For Paracetamol) and Figure-6B(For Tapentadol) to demonstrate the linearity of the proposed method.

From the data obtained which given in Table-4A (For Paracetamol) and Table-4B (For Tapentadol) the method was found to be linear within the proposed range.

Table 4B: Linearity studies for Tapentadol by proposed method

Linearity Study for Tapentadol		
% Level	**Conc. µg/mL**	**Area**
50	100	1060274
75	150	1607774
100	200	2139750
125	250	2658662
150	300	3217022
Slope		10729.0
Intercept		-9057.0
% Y-Intercept		-84.4
Residual Sum of Squares		10698.0
CC(r)		0.9999
RSQ(r2)		0.9999
LLD		3.29
LLQ		9.97

Figure 6B: Calibration curve for Tapentadol



CONCLUSION

An RP-HPLC method for simultaneous estimation of Tapentadol and Paracetamol was developed and validated as per ICH guidelines.

The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 643.50-1930.50µg/ml for Paracetamol and 100-300µg/mL for Tapentadol. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common

chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed.

We have developed a fast, simple and reliable analytical method for determination of Tapentadol and Paracetamol in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Paracetamol and Tapentadol It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of Tapentadol and Paracetamol in bulk, its different pharmaceutical dosage forms.

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