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Development of validated stability indicating assay method for tapentadol and paracetamol in bulk and combined 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 Hyperchrome C18 (250mm x 4.6, 5µm) in isocratic mode, with mobile phase containing 0.05phosphate buffer, acetonitrile (65:35 v/v) adjusted to pH 2.8 with ortho phosphoric acid. The flow rate was 1.0 mL/min and detection wavelength was 217 nm. Tapentadol, Paracetamol and their marketed formulation were exposed to acidic, alkaline, thermal, hydrolytic and oxidative, stress conditions, and the stressed samples were analyzed by the proposed method. The developed method was validated in terms of precision, robustness, recovery, and limits of detection and limit of quantitation. The described method exhibited a linear dynamic range of 1-6 µg/mL for Tapentadol and 6.5-39 µg/mL for Paracetamol. The calibration curves were found to be linear (r = 0.9961 for Tapentadol and r = 0.9978 for Paracetamol) over the range. Percent RSD of precision were found as 0.6137 for Tapentadol & 0.6158 for Paracetamol. The mean recovery was found to be 99.96% for PARA & 99.91% for TAP. The method was found to be suitable for analysis of Tapentadol and Paracetamol in presence of its degradation products.

Keywords: Stability Indicating, Tapentadol, paracetamol and forced degradation

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INTRODUCTION

Stability Indicating Method (SIM) is defined as a validated analytical procedure that accurately and precisely measure active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products.[1] Identification, quantification or purifying compounds of interest is major concern during HPLC analysis. The quality of a medicinal product varies as a function of time and under the influence of a variety of environmental factors, hence stability studies are carried out to monitor such effects on product quality. Method validation is the process of proving that an analytical method is acceptable for its intended purpose bulk drug and formulations.[2] The established method was validated according to ICH (International Conference on Harmonization) with respect to specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability.[3-4] Tapentadol (TAP) [Figure1] is chemically 3-[(1R, 2R)-3-(dimethyl amino)-1-ethyl-2-methylpropyl] phenol hydrochloride. It is centrally acting oral µ-opioid receptor agonist and also inhibits nor epinephrine and serotonin reuptake within the CNS. It is used in metastatic bone cancer, postsurgical dental pain and painful diabetic nephropathy.

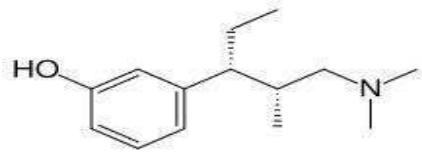

Figure 1: Structure of Tapentadol

Paracetamol(PARA) [Figure2] is chemically 4-Hydroxyacetanilide. It is used as Analgesic and Antipyretic.

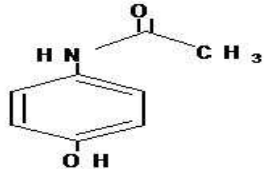

Figure 2: Structure of Paracetamol

Literature survey revealed that many analytical methods have been reported for the determination of Tapentadol and Paracetamol in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography either in single or in combined forms [5-11]. This study was designed to develop a simple, reliable and quantitative method for estimation of Tapentadol and Paracetamol in a relatively short time with high sensitivity along with stress stability studies. To establish the stability indicating nature of the method, forced degradation studies was performed on Exposed Standard and marketed formulation under stress conditions (thermal, photolytic, acid and basic hydrolytic and oxidative) and stressed samples were analyzed by the proposed method. The proposed LC method was able to separate both. Therefore, this method can be employed for the routine analysis of Tapentadol and Paracetamol in combined dosage form.

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Tapentadol and Paracetamol were kindly provided as gift sample from Glenmark pharmaceuticals (Mumbai). Commercial tablet formulation of Tapentadol and Paracetamol were procured from the local chemist shop. Water, acetonitrile and methanol used were of HPLC grade. Other chemicals were of either AR grade or GR grade.

2.2 Instrument

Chromatographic separation was performed on Analytical Technology Limited isocratic system consisted of hyperchrome ODS 5µ C_{18} column (250 X 4.6 mm), Uv-3000 detector and P-3000 Pump, Rheodyne injector with 20µL capacity. The mobile phase comprised of 0.05M Potassium Dihydrogen phosphate Buffer: Acetonitrile, pH 2.8(65:35) at flow rate 1.0 mL/min. The mobile phase was filtered through a 0.45µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature. The detection was monitored at 217nm.

HPLC METHOD DEVELOPMENT

Preparation of Mobile phase

7g Potassium Dihydrogen orthophosphate was dissolved in 1000 mL of water and Mixed, pH adjusted to 2.8 using ortho phosphoric acid, sonicated to degas the buffer.

The mobile phase was prepared by mixing Acetonitrile and Buffer in the ratio of 35:65v/v. The mobile phase was filtered through nylon 0.45µm membrane filter. The same mobile phase was used as diluents.

Preparation of solutions

i. Mix standard stock solution (A)

An accurately weighed quantities of Tapentadol (TAP) and Paracetamol (PARA) in the ratio of 6.5: 1 were transferred to a 50.0 mL volumetric flask, and volume was made up to the mark with mobile phase. A 1.0mL portion was further diluted to 25.0mL (Conc:20.0µg/mL TAP; 130.0µg/mL PARA).

ii. Mix working standard solution (B)
A 2.5 mL portion of the above stock solution A was further diluted up to 10.0 mL with mobile phase. (Conc: 5.0µg/mL and Conc: 32.5µg/mL)

Procedure for forced degradation study
Forced degradation of Exposed standard and marketed formulation was carried out under thermolytic, photolytic, acid /base hydrolytic and oxidative stress conditions. Thermal and photo-degradation of exposed standard and marketed formulation was carried out in solid state.

Solution state studies were carried out by using (0.5N HCL, 0.5N NaOH, Water, 3%H_2O_2) for acid, base, neutral and oxidative hydrolysis respectively. The exposed standard were refluxed for specified time as indicated, a (30min), b (60 min), c (90 min), d (120min), (150 min) and f (180min) and marketed formulation for 180 min. Then it was cooled to room temperature and mobile phase was added to each flask and volumes were adjusted up to the mark with mobile phase. The content in each flask were sonicated for 20min. All the samples were chromatographed using 20µL volume. The marketed formulation was also treated in same manner as that of exposed standard.

For thermal stress, exposed standard and Marketed formulation were placed in a controlled-temperature oven at 50 °C for 3 h. For photolytic stress, exposed standard and marketed formulation, both in solid state, were irradiated with UV radiation with peak intensities at 254 nm for 24 h and 48 h. For humidity stress, exposed standard and marketed formulation were kept in 75% RH at ambient temperature for period of 15 days. After the degradation, these stock solutions were prepared in mobile phase and then appropriately diluted with mobile phase to get concentration of 5µg/mL of TAP and 32.5µg/mL of PARA. (On label claim basis)

2. Application of proposed method for assay of marketed formulation
Twenty tablets were weighed and average weight was calculated. The tablet were triturated thoroughly and mixed. An accurately weighed quantity of tablet powder equivalent to 25.0 mg of TAP (~162.5 mg of PARA) was transferred to 50.0 mL volumetric flask. The mobile phase was added to the flask, shaken and volume was made up to the mark. The content was sonicated for 20 minutes and filtered through whatmann filter paper (no.41). A 1.0 mL portion of the filtrate was diluted to 25.0 mL with mobile phase. A 2.5 mL portion of this solution was further diluted to 10.0 mL with mobile phase. (Conc. of TAP is 5.0µg/mL and Conc. of PARA is 32.5µg/mL). Five such samples were prepared. The 20 µL volume of the final diluted solution were injected separately, the representative chromatograms was recorded.

RESULTS AND DISCUSSION

3.1 Development of validated stability indicating method
To develop a precise, accurate, specific and suitable stability indicating RP-HPLC method for the simultaneous estimation of TAP and PARA, different mobile phases were employed and proposed chromatographic condition was found appropriate for the quantitative determination in the presence of degradation products and best for analysis. The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

Optimized chromatographic conditions
Column: Hyperchrome-ODS 5 µ C18 column (250 X 4.6mm), Mobile Phase: Acetonitrile and Buffer pH 2.8 (35:65 v/v), Detection Wavelength: 217nm, Flow rate: 1.0 mL/min, Temperature: Ambient: 28-30°C, Injection volume: 20µL

A 20µL solution of above mix standard was injected through manual injector and chromatogram was recorded using final mobile phase. A chromatogram for blank and standard of TAP and PARA so recorded in shown in fig 3a-b

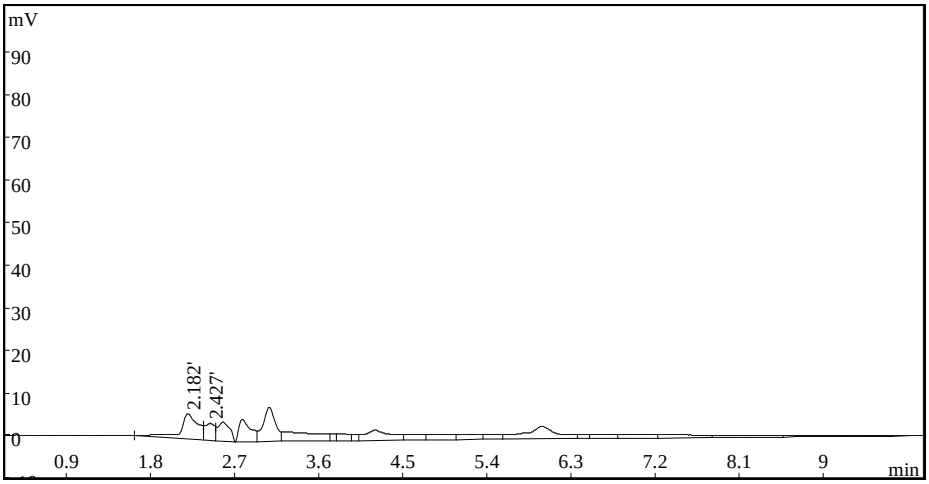

Fig 3a: **Chromatogram of Blank**

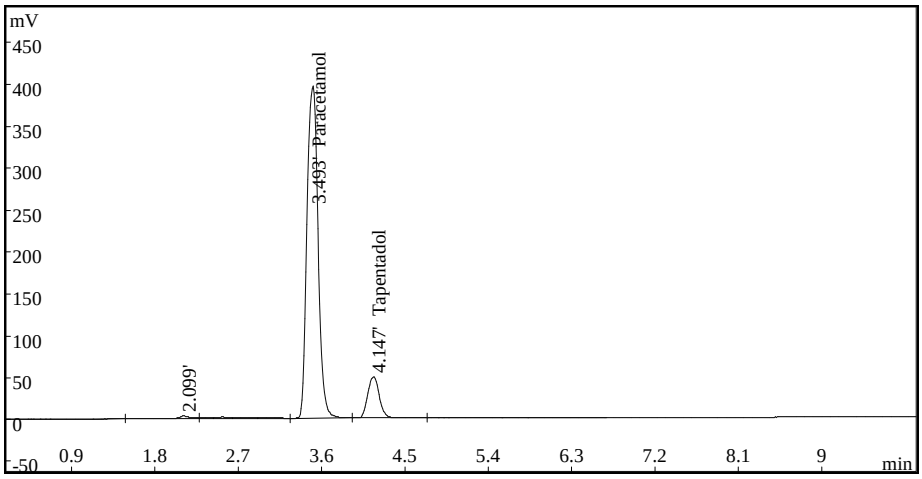

Fig 3b: Chromatogram of drugs under final chromatographic condition

Table 1: Observation of System Suitability Parameters

Sr. No.	Standard Weight Taken(mg)		A.U.C of PARA (mV)	A.U.C of TAP (mv)
	PARA	TAP		
1	~65.0	~25.0	393890	2790605
2			395149	2792744
3			395011	2793146
4			394922	2791322
5			393772	2789644
6			393921	2790064
Mean			394435.8	2791254
±S.D.			655.4889	1430.469
%RSD			0.16618	0.05124
Theoretical plate/column			3538	3414
Retention time			3.493	4.147
Asymmetry			1.1	1.07
Resolution				2.77

3.2 Study of system suitability parameters

After equilibration of column with mobile phase, five replicate injections of 20µL solution of (C) was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in Table 1.

3.3 Study of Linearity

The graphs of concentration of drug vs. area under curve were plotted and the correlation coefficient was found to be (r=0.9961) for TAP and0.997 for PARA drug.

3.4 Forced degradation study

3.4.1 Solution State Stability

a. Alkaline hydrolysis (0.5 N NaOH)

The study of chromatogram (fig. 4a-b) reveals that the drugs were was found to be falsely susceptible to acidic degradation. Paracetamol and tapentadol were found to degrade around 3.62% and 19.9% in exposed standard while 3.32 % and 17.08 % in marketed formulation. No additional peaks were seen in the chromatograms

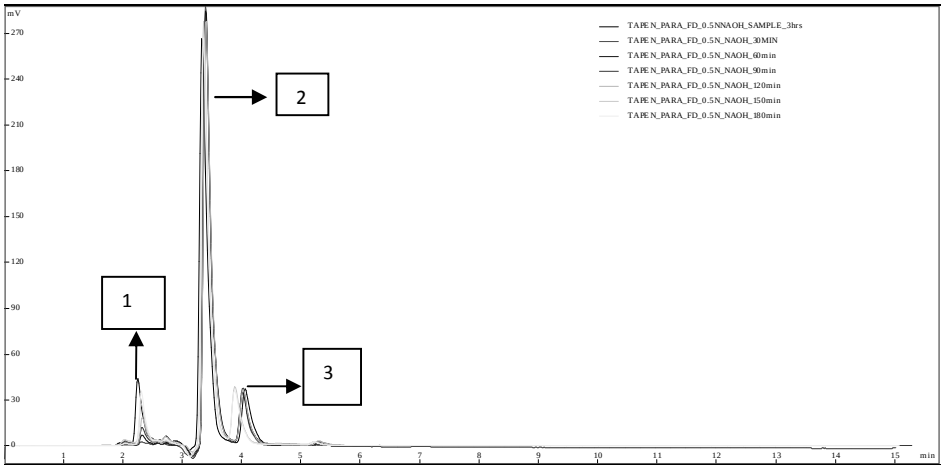

Fig 4a: Overlain chromatogram for exposed standard Under Alkali hydrolysis

1) Blank 2) Paracetamol 3) Tapentadol

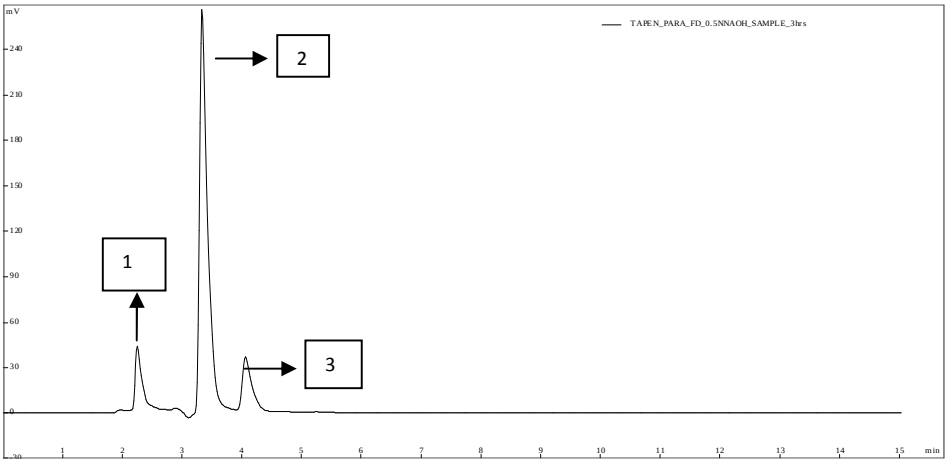

Fig 4b: Chromatogram for marketed formulation under Alkali hydrolysis

1) Blank 2) Paracetamol 3)Tapentadol

b. Acidic hydrolysis (0.5N HCl):

The study of chromatogram (fig. 5a-b) reveals that the drugs were found to be falsely susceptible to acidic degradation. Paracetamol and tapentadol were found to degrade around 15.54% and 3.81% in exposed standard while and 13.06 % and 3.32% in marketed formulation. No additional peaks were seen in the chromatograms.

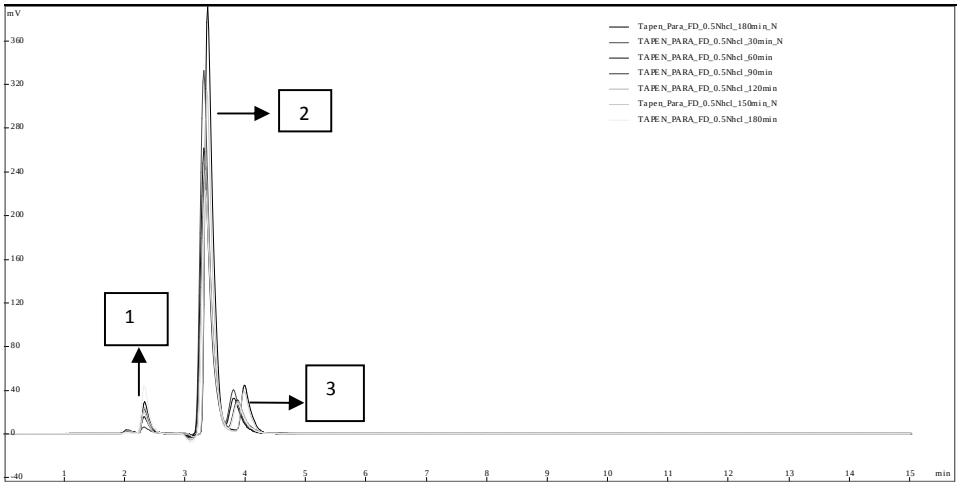

Fig 5a: Overlain chromatogram for exposed standard under acid hydrolysis
1) Blank 2) Paracetamol 3)Tapentadol

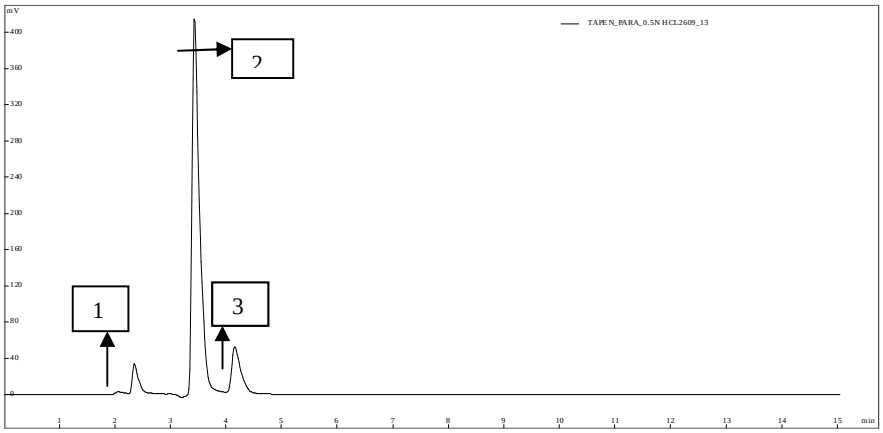

Fig 5 b: Chromatogram for marketed formulation under Acid Hydrolysis
1) Blank 2) Paracetamol 3) Tapentadol

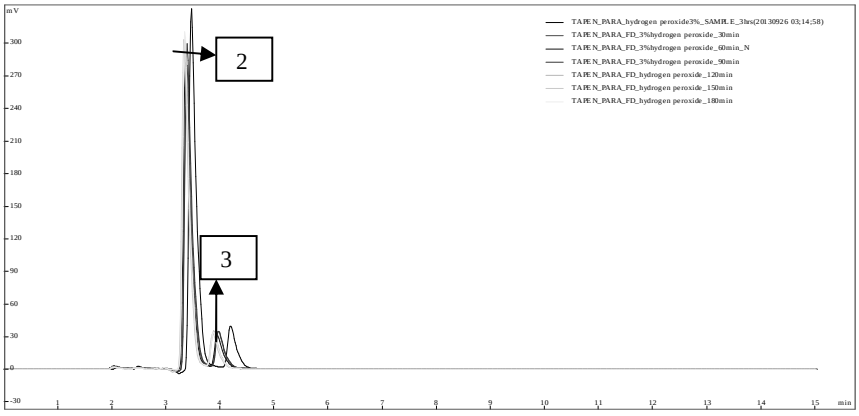

Fig6a : Overlain Chromatogram for exposed standard under Oxidative hydrolysis
2) Paracetamol 3)Tapentadol

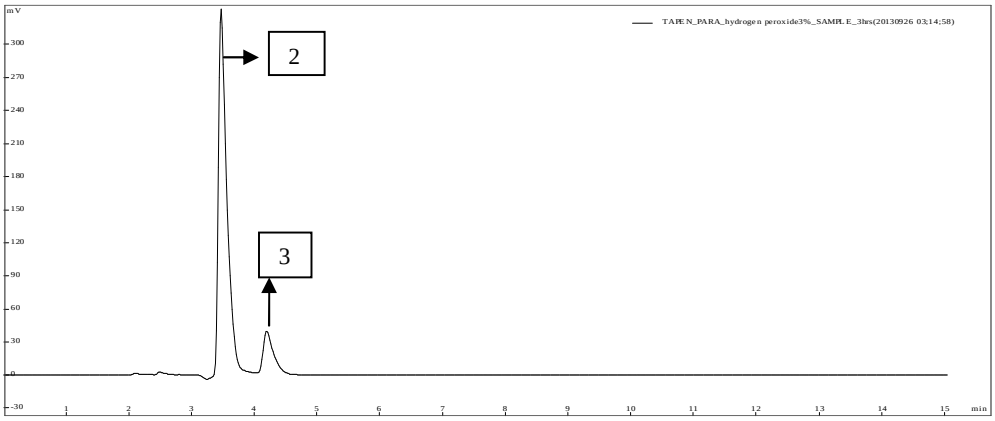

Fig 6b: Chromatogram for marketed formulation under Oxidative Hydrolysis
2)Paracetamol 3)Tapentadol

c. Oxidative hydrolysis (3 % H_2O_2)

The study of chromatogram (fig. 6 a-b) revealed that the drugs were found to be softly degraded under oxidative hydrolysis. Paracetamol and tapentadol were found to degrade around 4.04% and 2.76% in exposed standard while 3.32% and 2.5% in marketed formulation. No additional peaks were seen in the chromatograms.

d. Neutral hydrolysis (distilled water)

The study of chromatogram (fig. 7a- b) revealed that the drugs were found to be softly degraded under neutral condition. Paracetamol and tapentadol were found to degrade around 2.17% and 1.55% in exposed standard while 2.98% and 3.66% in marketed formulation. No additional peaks were seen in the chromatograms.

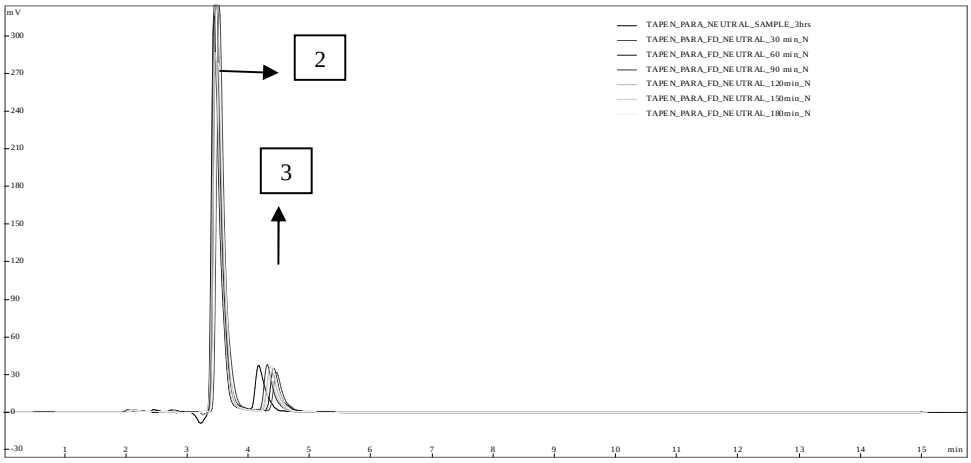

Fig 7a: Overlain Chromatogram for exposed standard under Neutral hydrolysis
2)Paracetamol 3)Tapentadol

3.4.2 Solid State Stability Studies

i) Humidity Study (40ºC/75% RH)

Exposed Drug & Marketed formulation (In powder form)

The study of the chromatogram (Fig. 8a-b) reveals that no additional peak was observed on exposure to 40ºC/75% RH of exposed standard and marketed formulation for a period of 15 days. The area under the curve was decreased on 15th day but no additional peaks were seen in chromatogram. The results indicated that, on 15th day paracetamol and tapentadol were found to degrade around 35% and 27% in exposed standard while 29% and 18% in marketed formulation. The degradation was found to be overdone.

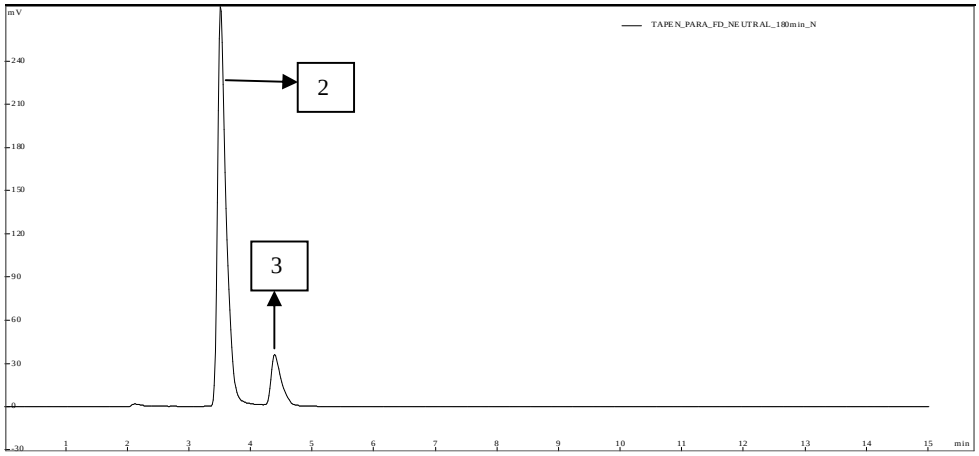

Fig 7b: Chromatogram for marketed formulation under Neutral hydrolysis
2)Paracetamol 3)Tapentadol

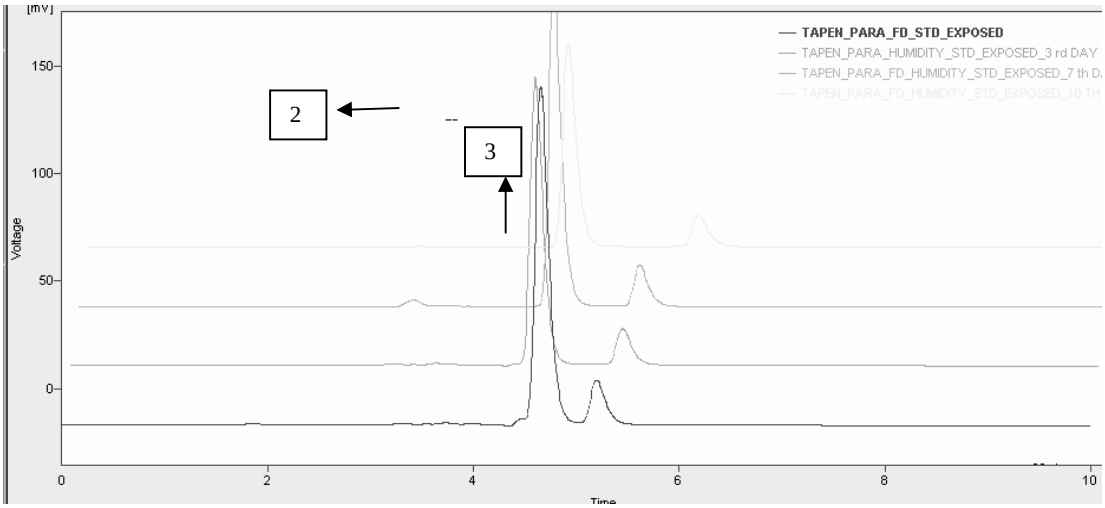

Fig 8a: Overlain Chromatogram for exposed standard under humidity studies
2)Paracetamol 3)Tapentadol

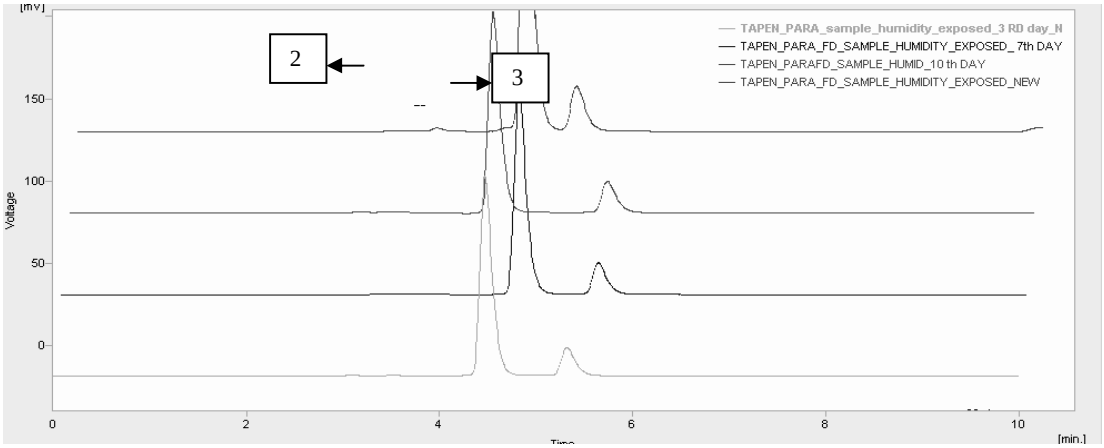

Fig 8b: Overlain Chromatogram for marketed formulation under humidity studies
2)Paracetamol 3)Tapentadol

Photostability studies

ii) UV Light

When exposed standard and marketed formulation were kept in UV light at 254 nm for a period of 2 days, the study of chromatogram (Fig.9a-b) reveals that there was no additional peak were seen in the chromatogram. After 48 hrs both exposed standard and marketed formulation was found to be false susceptible to degradation by UV light.

The exposed standard and marketed formulation were found to degrade around 4.4%, 2.38 % and 4.89%, 4.79 % of paracetamol and tapentadol respectively.

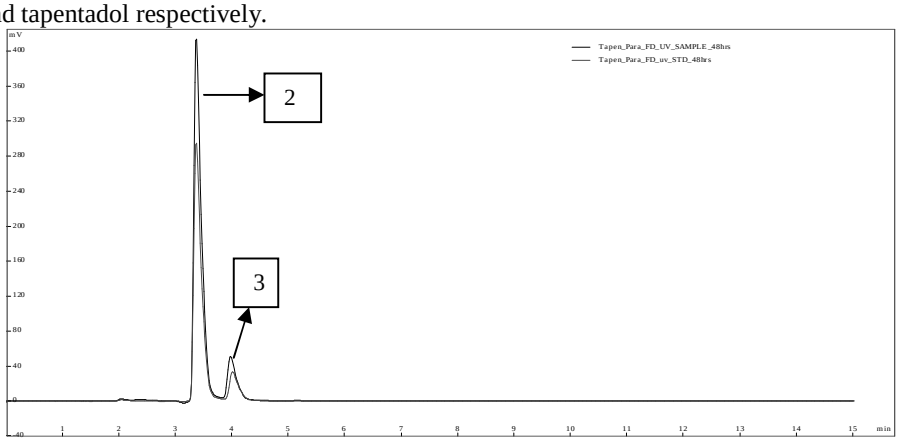

Fig 9a: Overlain Chromatogram for exposed standard under UV studies
2)Paracetamol 3)Tapentadol

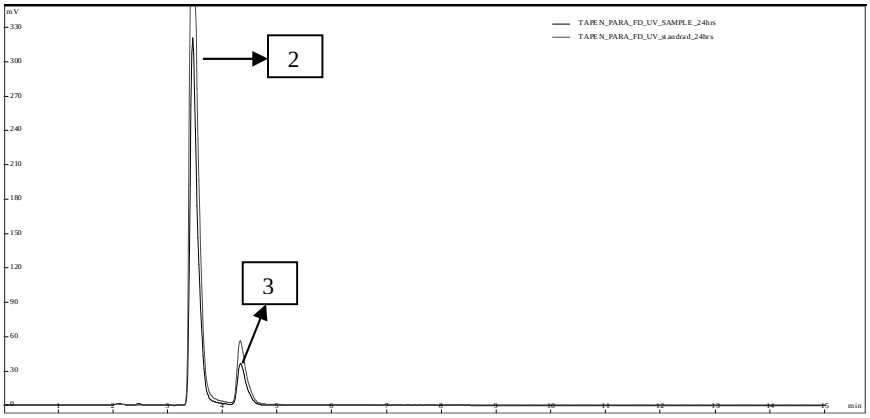

Fig 9b: Overlain Chromatogram for marketed formulation under UV studies
2)Paracetamol 3)Tapentadol

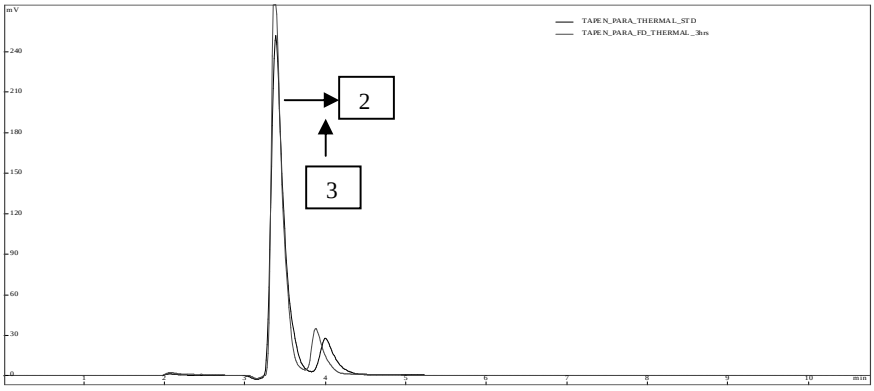

Fig 10: Overlain Chromatogram for exposed standard & marketed formulation for thermal studies
2)Paracetamol 3)Tapentadol

iii) Thermal studies (50°C)

When powdered sample was exposed to dry temperature at 50ºC for a 3hours, there was no significant change in the area under curve for samples, no additional peaks were observed in the chromatogram (Fig. 10) The results indicated that, on 3rd hour exposed standard and marketed formulation does not degrade i.e. both drugs were found to be soft susceptible to thermal degradation.

Summary of forced degradation studies are shown in Table 2

Table 2: Summary of forced degradation studies

Sr. No.	Stressed Condition	% Undegraded drug			
		Exposed standard		Marketed formulation	
		PARA	TAP	PARA	TAP
1	Acidic reflux (3h)/0.5N HCL	84.46	96.68	86.94	96.19
2	Alkaline reflux (3h)/0.5N NAOH	96.38	80.10	96.68	82.92
3	Neutral reflux (3h)/Water	97.83	96.34	98.45	97.02
4	Oxidation reflux (3h)/3 %H2O2	95.96	97.24	97.22	97.5
5	Humidity studies(15days)/ 75% RH /40^{0}c	65.07	73.23	71.21	82.62
6	UV light (2 days)/254nm	95.6	95.11	97.62	95.21
7	Thermal studies (3h)/50^{0}c	99.95	100.06	100.54	100.53

Application of proposed method for assay of marketed formulation

The 20 µL volume of the final diluted solution were injected separately, the representative chromatograms was recorded and shown in fig. 11.

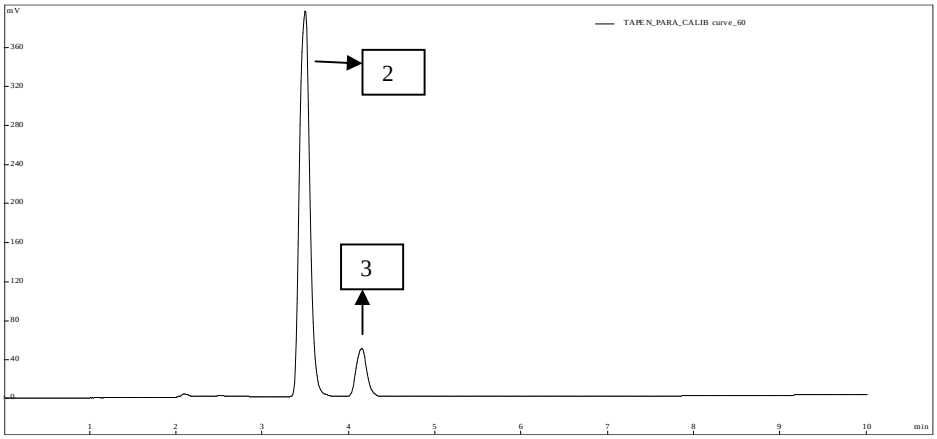

Fig 11: Chromatogram of marketed formulation 2)Paracetamol 3)Tapentadol

Table 3: Results of estimation in marketed formulation

Sr. No.	AUC of Sample (mV)		Amt. Estimated in Avg. wt. of Tab(mg)		% Label claim	
	PARA	TAP	PARA	TAP	PARA	TAP
1	2900031	394690	328.67	49.74	101.13	99.49
2	2884962	392640	328.34	49.69	101.03	99.39
3	2864887	391091	328.96	49.94	101.22	99.88
4	2843197	393820	326.78	50.33	100.55	100.67
5	2799982	389980	324.35	49.92	99.8	99.85
6	2824890	387197	324.9	49.52	99.67	99.04
				Mean	100.62	99.72
				± SD	0.6145	0.5602
				%RSD	0.6107	0.5617

Method Validation

The method was validated as per the guidelines in terms of parameters like, precision, accuracy (recovery studies), system suitability parameters, linearity and range etc.

i. Precision

Precision of proposed method was ascertained by replicate analysis of homogeneous samples. Precision of any analytical method is expressed as SD and RSD of series of measurements. The mean percent labeled claim was found to be 100.61 for PARA and 99.72 for TAP. The % RSD was found to be 0.6145 for PARA & 0.5602 for TAP. Results shown in Table 3.

ii. Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. The mean % recovery was found to be 99.96 for PARA & 99.91 % for TAP. The % RSD was found to be 0.1512 for PARA & 0.397 for TAP. Results of recovery (accuracy) study are summarized in Table 4.

Table 4: Results of Accuracy Study

Sr. No.	Amt. of pure drug added (mg)		Total Amt. of Drug Estimated (mg)		Amount Recovered (mg)		% Recovery	
	PARA	TAP	PARA	TAP	PARA	TAP	PARA	TAP
1	98.42	15.33	262.55	40.27	98.5	15.26	100.08	99.73
2	131.1	20.4	294.24	45.28	130.77	20.36	99.74	99.8
3	162.02	25.85	326.58	51.06	162.05	25.97	100.01	100.46
4	194.4	30.05	258.58	54.82	195.44	25.95	100.02	99.66
						Mean	99.962	99.912
						± SD	0.1515	0.3694
						%RSD	0.1515	0.3697

iii. Ruggedness

Intermediate precision (Intraday and Interday) shows the % Label claim values within limits (% RSD not more than 2). The method was found to be precise. The ruggedness studies were carried out using different analyst variation. The results of intermediate precision parameter are shown in Table 5.

Table 5: Results of Intermediate precision

Parameters	Mean % label claim ± S.D. of PARA	Mean % label claim ± S.D. of TAP
Different Analyst (n=3)	100.01 ± 0.36	100.22 ± 0.14
Intraday Variation (n=4)	98.97 ± 0.60	99.17 ± 0.61
Interday Variation (n=3)	96.01 ± 2.79	96.01 ± 1.76

iv. Linearity and range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110 and 120% of label claim of TAP were taken and dilutions were made as described under marketed formulation. Then, each solution was injected and chromatograms were recorded.

The correlation coefficient was found to be 0.997 for paracetamol and 0.996 for tapentadol.

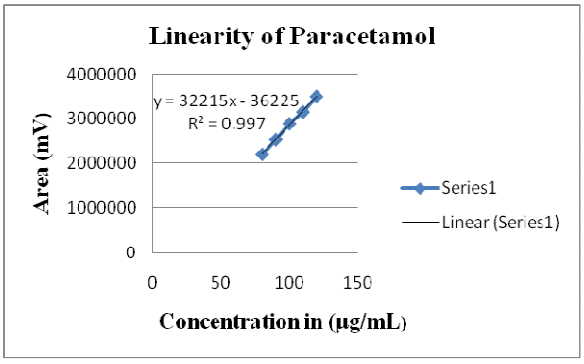

Fig 12: Linearity of Paracetamol

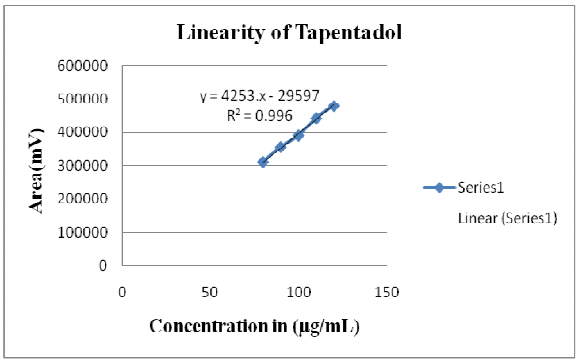

Fig 13: Linearity of Tapentadol

v. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The system suitability parameter was evaluated for each varied condition. The amount of TAP and PARA was calculated from sample solution in each varied condition. Results are shown in Table 6**.** The results of above study shown that the method were robust under varied conditions.

Table 6: Observation and result of Robustness study

Sr.No	Deliberate Changes	R.T.		Asymmetry		% Labelled claim	
		PARA	TAP	PARA	TAP	PARA	TAP
1	Standard Condition	3.493	4.147	1.07	1.17	100.55	99.85
2	Change in flow rate (1.1 ml)	3.112	3.824	1.62	1.74	101.67	100.35
3	Change in flow rate (0.9ml)	3.811	4.621	1.80	1.68	101.77	100.48
4	Change in Wavelength (212nm)	4.015	3.374	1.34	1.65	98.54	98.08
5	Change in Wavelength (222nm)	3.969	3.330	1.48	1.55	99.98	97.92
6	Change in pH (2.6)	3.392	3.990	1.72	1.41	100.21	99.26
7	Change in pH (3.0)	3.637	4.024	1.58	1.35	99.75	98.98
	SD	0.3265	0.2283	0.2477	0.3132	1.1248	1.0710
	Overall SD	0.5519					

vi. Limit of Detection and Limit of Quantification

The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ .The results are shown in Table 7. The results showed good sensitivity of method.

Table No 7: Result of LOD and LOQ

Sr. No.	LOD (µg/mL)		LOQ (µg/mL)	
	PARA	TAP	PARA	TAP
1	40.27	6.184	122.05	18.74

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Paracetamol & Tapentadol from pure drug and its dosage form. The method provides selective quantification of both drugs without interference from excipients affirming its stability indicating nature. The method was completely validated to ensure the compliance in accordance with ICH guidelines, and satisfactory results were obtained for all the method characteristics tested. 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. Also the low detection and quantitation limits indicate high sensitivity of method. The method can be adopted for the routine analysis of Paracetamol and Tapentadol in pure form as well as its dosage form.

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REFRENCES

[1] M. Bakshi, S. Singh S, *J. Pharm. and biomed Anal.*, **2002**, 28, 1011–1040
[2] R. Singh, *Journal of Pharm Educ Res.*, **2013**, 4, 26-33.
[3] ICH-Harmonised Tripartite Guideline, Stability Testing of New Drug Substances and Products (Second Revision), FDA, Q1A (R2), Nov. **2003**, 225, 657-678.
[4] ICH-Harmonised Tripartite Guideline, Photostability Testing of New Drug Sub-stances and Products , FDA, Q1B, 95, **1997**,62, 27115-27122.
[5] http://www.who.int/entity/medicines/areas/quality_safety/5.2Tapentadolprereview. Pdf
[6] G. Tayal, A. Grewal, Mittal R, Bhatia N, *Journal of Anaesth. Clinical Pharma*, **2009**, 25, 463-466.
[7] Indian Pharmacopoeia Government of India, Ministry of Health and Family Welfare, Controller of India, New Delhi,7th edition, Vol. III, **2010**, 107.
[8] USP-NF, Validation of Compendial Procedures, General Chapters, Vol. I, **2010**, 734-736.

[9] http://www.pharmweb.net/pwmirror/pwy/paracetamol/pharmwebpicmechs. html.
[10] P.T. Bhatasana, A.R. Parmar, *Der Pharma. Sinica*, **2012**, 3, 422-426.
[11] R. Ganji, D. Ramachandran, *Drug Invent Today*, **2012**, 4, 227-234.
[12] G. Saravanan, Y. Mohammad, *Indian Journal of Pharma. Sci*, **2010**, 2, 75-79.
[13] D.N. Bhakhar, A.R. Parmar, *Inventi Rapid: Pharma Analysis & Quality Assurance*, **2013**, 20, 56-59.
[14] R. Thimma, M. Ramesh, *Asian Journal of Research in Chemistry*, **2012**, 5, 1255-1261.
[15] M.S. Charde, A.A. Patil, J. Kumar, A.S. Welankiwar, and R.D. Chakole, *International Journal of Phytopharmacy*, **2013**, 3 , 90-98.
[16] S. Kathirvel, S. Venkata, D.G. Satyanarayana, *Journal of Chemistry*, **2013**, 10, 1-9.