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Research Article

Chromatographic development of validated analytical method for the estimation of tapentadol and paracetamol in combined dosage form

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Abstract

A simple, sensitive an isocratic RP-HPLC method for the estimation of TAP (Tapentadol) and PARA (Paracetamol) in combined dosage form using Inertsil ODS C-18 column (250×4.6 mm, 5 µ) in an isocratic mode with mobile phase comprising Buffer (1mL TEA) : ACN : MeOH in the ratio of (75:20:5 v/v/v). The flow rate was 1.2 mL/ min and effluent was monitored at 220 nm. The retention times were found to be 6.88 min for TAP and 3.78 min for PARA. The assay exhibited a linear dynamic range of 11.89- 28.55 µg/mL for TAP and 64.95- 155.90 µg/mL for PARA .The calibration curves were linear (r = 0.999 for TAP and r = 0.9996 for PARA) over the entire linear range. Recovery was found to be 99.98 % ± 1.259 for TAP and 100.0 % ± 0.8625 for PARA. % RSD of system precision were observed 0.080 for TAP & 0.069 for PARA and % RSD of method precision was found 0.084 and 0.384 for TAP and PARA respectively. The observed values for Ruggedness studies were 0.421 % and 1.019 % for TAP and PARA respectively.
Key Words: Tapentadol, Paracetamol, High Pressure Liquid Chromatography, Validation

1.Introduction

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products. Method validation is the process of proving that an analytical method is acceptable for its intended purpose[1]. The parameters for method validation as defined by ICH (International Conference on Harmonization) guidelines are Accuracy, Precision, Specificity, Limit of Detection, Limit of Quantitation, Linearity, Range, Robustness, Ruggedness. The purpose of the stability studies is to ascertain how the quality of a medicinal product varies as a function of time and under the influence of a variety of environmental factors. The ICH guidelines 'Stability testing of new drug substances and products ' (QIA) requires that stress testing should be carried out to elucidate the substance. It suggests that the degradation product that are formed under the variety of condition should include the effect of temperature, humidity where appropriate, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH value. The study of the effect of temperature is suggested to be done in 10^{o}C increment above the accelerated temperature test condition (e.g. 50^{o}C, 60^{o}C etc.) and that of humidity at a level of 75 % or greater. No details are however provided for the study of oxidation, photolysis and hydrolysis at different pH values[2-4]. Tapentadol [Figure1] is chemically 3-[(1R, 2R)-3-(dimethyl amino)-1-ethyl-2-methylpropyl] phenol hydrochloride. It is a centrally-acting, oral µ-opioid receptor agonist which also inhibits norepinephrine and serotonin reuptake within the CNS. It is used in metastatic bone cancer, postsurgical dental pain, painful diabetic

nephropathy. It is Freely Soluble in water, 0.1N HCl, simulated intestinal fluid and at controlled room temp (22^0C,77^0F) protected from moisture.[5,6] Paracetamol[Figure2] is chemicaly 4-Hydroxyacetanilide. It is used as Analgesic and Antipyretic. It is insoluble in water, very soluble in ethanol.[7-9] In literature, many analytical methods have been reported for estimations of Tapentadol And Paracetamol individually from biological fluids.[10-14] Therefore, the present aim was undertaken to develop simple, accurate, precise and rapid and RP-HPLC method for determination TAP and PARA in a combined dosage form .

Figure 1 : Structure of Tapentadol

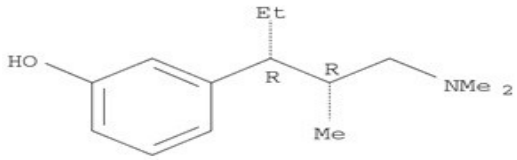
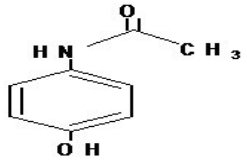


Figure 2 : Structure of Paracetamol



2. Methods and Materials

2.1 Chemicals and Reagents: All the solvents and chemicals used were of HPLC and analytical grade. Mili Q water and 0.45 µm Teflon filter was used throughout the experimental work. The gift drug samples of TAP and PARA were provided by Glenmark R & D, Sinnar. The tablet formulation of TAP & PARA (Vorth TP Plus, Manufactured by Glenmark Pharmaceuticals) was purchased from the market . Chemicals and Reagents Used are Hydrogen Peroxide 30%, Ortho-Phosphoric acid, Concentrated Hydrochloric Acid, Potassium Dihydrogen Orthophosphate, Sodium Hydroxide Pellets, Acetonitrile, Water, Methanol, Triethylamine.

2.2 Instrument: The chromatographic separation performed using Waters HPLC System with PDA detector, model 2996. Software used to monitor was Empower Pro and Quaternary pump is applied. Analytical balance is used, Make Sartorious (Model AB - 20.04). pH meter was also used, Labindia Make, Model pH System 362.

2.3 Preparation of Mobile Phase: The dilution was prepared by mixing Buffer solution, ACN & MeOH in the ratio (75:20:05 v/v), degas it with ultrasonic bath & use.

2.4 Preparation of Diluent: The dilution was prepared by mixing Buffer solution, ACN & MeOH in the ratio of (50:40:10 v/v), degas it with ultrasonic bath & use.

2.5 Preparation of Buffer (0.025 M): Dissolve 1.36 gm of monobasic KH_2PO_4 in 100.0 mL of water, and add 1.0 mL of TEA, adjust pH to 3.0 with dilute OPA. Filter it through nylon membrane filter of 0.45 µm.

2.6 Selection of Analytical Wavelength: The absorbance of TAP (20.0 µg/mL) and PARA (130.0 µg/mL) solutions were found highest at 220 nm. Therefore, 220 nm was selected as a wavelength for the estimation of drugs. The Overlain Spectra of TAP &PARA was recorded and shown in the **figure 3**.

2.7 Analysis of Physical Laboratory Mixture

2.7.1 Preparation of Standard Stock Solution A: Weigh accurately 29.11 mg of TAP HCl working standard (equivalent to 25.0 mg of TAP) and transfer it into 100.0 mL dry volumetric flask, add 70.0 mL of diluent, keep the flask in ultrasonic bath for 5 Min to dissolve the drug completely and make up the volume with diluent. (Conc. of TAP is 250.0 µg/mL)

2.7.2 Preparation of Standard Stock Solution B: Weigh accurately 65.0 mg of PARA working standard and transfer it into 100.0 mL dry volumetric flask, add 70.0 mL of diluent, keep the flask in ultrasonic bath for 5 Min to dissolve the drug completely and make up the volume with diluent. (Conc. of PARA is 650.0 µg/mL)

2.7.3 Mix Standard Solution: Pipette out 4.0 mL of solution A & 10.0 mL of solution B into 50.0 mL volumetric flask & make up the volume with water. (Conc. of TAP is 20.0 µg/mL and Conc. of PARA is 130.0 µg/mL).

2.7.4 Preparation of Sample Solution: Weigh accurately 29.11 mg of TAP HCl (equivalent to 25.0 mg of TAP) & 65.0 mg of PARA into 500.0 mL volumetric flask add 70.0 mL of diluent, shake it vigorously to dissolve the drug completely and make up the volume with diluent. It was further diluted to get concentration of 20 µg/mL of TAP and 130 µg/mL of PARA.. The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtain the concentration. The amount of each drug estimated in laboratory mixture was calculated using following formula-

$$\% \text{ Estimation} = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times 100$$

Where, At = Area count for sample solution; As = Area count for standard solution; Ds = Dilution factor for standard; Dt = Dilution factor for sample; Ws = Weight of standard (mg); Wt = Weight of sample (mg)

The results are shown in **Figure6** & discuss in the **table2.**

2.8 Analysis of Marketed Formulation

2.8.1 Preparation of Sample Solution: Weigh accurately 20 tablets and calculate the average weight then crushed the tablets into fine powder. Transfer 5 tablets into 500.0 mL volumetric flask and sonicate it for 15-20 Min with intermittent shaking and make up the volume with diluent. Centrifuge, the portion of solution at 3000 rpm for 10 Min to get a clear solution. (Conc. of TAP is 500.0 µg/mL and Conc. of PARA is 3250.0 µg/mL)

Pipette out 2.0 mL of above solution in 50.0 mL volumetric flask & make up the volume with water. Filter the above solution through 0.45 µm Teflon filer paper. (Conc. of TAP is 20.0 µg/mL and Conc. of PARA is 130.0 µg/mL).

The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The % Label Claims of TAP and PARA were calculated by comparing a sample peak with that of standard.

a) Amount of TAP HCl equivalent to TAP in mg per tablet was calculated by using following formula

$$\% \text{ Label claim} = \frac{Aspl}{Astd} \times \frac{Wstd}{100} \times \frac{4}{50} \times \frac{500}{Wspl} \times \frac{50}{2} \times \frac{\%P}{100} \times \frac{221.34}{257.8} \times A$$

b) Amount of PARA in mg per tablet was calculated by using following formula

$$\% \text{ Label claim} = \frac{Aspl}{Astd} \times \frac{Wstd}{100} \times \frac{10}{50} \times \frac{500}{Wspl} \times \frac{50}{2} \times \frac{\%P}{100} \times A$$

Where, Aspl = Area count for Sample solution.; Astd = Area count for Standard solution.; Wstd = Weight of Standard in mg; Wspl = Weight of Sample in mg; LC = Label claim; A = Average weight of tablet in mg; %P = Potency of Standard; 221.34 = Molecular wt of TAP; 257.8 = Molecular wt of TAP HCl

The results are shown in **Figure7** & discuss in the **table3.**

2.9 Optimization of Chromatographic Condition for Estimation of Drugs: The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing mixture of TAP & PARA was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through 0.45 µm Teflon filter.

Finally, the optimal composition of the mobile phase, KH_2PO_4 buffer with 1.0 mL of TEA (pH adjusted to 3.0 with OPA): ACN: MeOH in the ratio of 75:20:05 was selected. It gave high resolution of TAP and PARA with minimal tailing.

2.9.1 Caliberation Curves for TAP & PARA: Aliquots of TAP and PARA standard solutions were transferred into 100.0 mL volumetric flasks. The volume was adjusted to the mark with diluent to obtain concentrations in the range of 80%- 120%. The graph of peak area obtained verses respective concentration was plotted. The mean area was calculated.

2.10 System Suitability Test: System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be carried out. It is performed to ensure that the system is operating properly and read to deliver results with acceptable accuracy and precision. The tests were performed by collecting data from five replicate injections of standard solutions.

2.11 Validation Parameters [15-17]

2.11.1. Linearity: A series of standard preparations of TAP and PARA working standard were prepared over a range of 50% to 120% of the working standard concentration of TAP and PARA in TAP and PARA tablet. Since the working standard concentration was 20 µg/mL and 130 µg/mL for TAP and PARA, the proposed ranges were about 11.89 µg/mL to 28.55 µg/mL and 64.95 µg/mL to 155.90 µg/mL for TAP and PARA respectively. Plot a graph of peak response against concentration. Determine the correlation coefficient.

2.11.2. Accuracy: Placebo of TAP and PARA tablet was spiked with TAP and PARA drug corresponding to level 80, 100 and 120 % of label claim in triplicate (in total nine determination). Acceptance Criteria: Mean recovery should be in the range of 98-102%. The Relative Standard Deviation should not be more than 2.0%

2.11.3. Precision: Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation.

2.11.4 Ruggedness: It is the degree of reproducibility of the test result obtained by analysis of samples, under a variety

of condition such as different lab, analyst, instrument, lots of reagents, elapsed time, different time, temp, days etc . Six sample solution of same lot (as used in 3.2) of TAP & PARA tablets were analysed by different analyst using same make of different HPLC column, on different day & HPLC as per described method.

2.11.5 Specificity (Force Degradation Study): Specificity is an ability to measures accurately and specifically the analyte of interest in the other components that may be expected to be present in the sample matrix. The result was shown in the **Table1**.

2.11.6. Robustness: It is the measure of capacity of the method to remain unaffected by small but deliberate variation in method parameter and provides an indication of its reliability under normal usage.

Standard solution, placebo solution and sample solution in triplicate were prepared. The sample along with standard and placebo were injected under different chromatographic conditions as shown below.

- Changes in organic phase composition. (±2%)
- Changes in column oven temperature. (±5°C)
- Changes in flow rate. (±0.20 ml/min)

2.12 Stability of Analytical Solution: Prepare standard and sample solutions as per the method and inject one standard and sample preparation initially at 0 hour and after specified time-intervals i.e. after 4 hours, 8 hours and 12 hours. Monitor the pattern of chromatogram at the pre-determined intervals and compare it against the initial pattern. Calculate the assay at each time interval. The stability of analytical solution is checked for drugs upto 12 hours.

3. Results and Disscussion

3.1 Optimization of Chromatographic Condition for Estimation of Drug

Column	: Inertsil ODS (250×4.6 mm, 5 µ)
Flow Rate	: 1.2 mL/Min
Wavelength	: 220 nm
Injection Volume	: 20.0 µL
Column Oven Temperature	: 30ºC
Run Time	: 12 Min
Mobile Phase	: Buffer (1mL TEA) : ACN : MeOH (75:20:5 v/v/v)
pH	: 3.0

Proper peak shape was observed for both TAP & PARA and system suitability parameters was observed within the limits. The result was shown in the **Figure4**.

3.2 System Suitability Test: % RSD of five replicate injections of TAP & PARA was found to be 0.08 & 0.07 respectively. Theoretical plates for TAP & PARA was found to be 7478.6 & 7072.8 respectively. Tailing factor for TAP & PARA was found to be 1.141& 1.097 respectively This indicates that system suitability parameters for proposed method were found to be within the limit. The result was shown in the **Figure 5.**

3.3 Validation Parameters

3.3.1. Linearity: Linearity regression coefficients were observed 0.9991 & 0.9996 for TAP & PARA respectively. The observed values were within the acceptance criteria for the Linearity of the method. Therefore, the proposed HPLC method for the determination of TAP and PARA in a tablet was found to be linear. The linearity was shown in the **figure8** & **9**.

3.3.2. Accuracy: % mean recoveries were found 99.98 % with % RSD 1.259 for TAP & 100.0 with % RSD 0.8625 for PARA. This showed that, the proposed HPLC method for the determination of TAP and PARA in a tablet was found to be accurate. The result was shown in the **figure11**.

3.3.3. Precision

3.3.3.1 system precision: % RSD of system precision was observed 0.080 for TAP & 0.069 for PARA. The observed values were within the acceptance criteria for System Precision study. This showed that, the proposed HPLC method for the determination of TAP and PARA in a tablet was found to be precise. The result was shown in the **figure 12**.

3.3.3.2 Method Precision (Intraday precision): % RSD of method precision was found 0.084 and 0.384 for TAP and PARA respectively. The observed values were within the acceptance criteria for Intraday Precision study .Therefore, the proposed HPLC method for the determination of TAP and PARA in a tablet was found to be precise. The result was shown in the **Figure13**.

3. 4. Ruggedness: % RSD was observed 0.421 and 1.019 for TAP and PARA respectively. The observed values were within the acceptance criteria for Ruggedness study. This showed that, the proposed HPLC method for the

determination of TAP and PARA in a tablet was found to be rugged. The result was shown in the **Figure 14**.

3.5. Specificity: % degradation for TAP was observed 16.5 % in base & that of PARA was 19.1% in acid. This showed that, the proposed HPLC method for the determination of TAP and PARA in a tablet was found to be specific. The result was shown in the **Figure 15 & 16, discuss in the table 4.**

3.6. Robustness: % R.S.D. of six replicate injections (Low temp, High temp) was observed 0.6836, 0.827 for TAP & 0.700, 0.292 for PARA .% R.S.D. of six replicate injections (Low temp, High temp) was within the acceptance criteria. This showed that, the proposed HPLC method for the determination of TAP & PARA in a tablet was found to be robust**.**

Figure 3 : Overlain Spectra of TAP &PARA

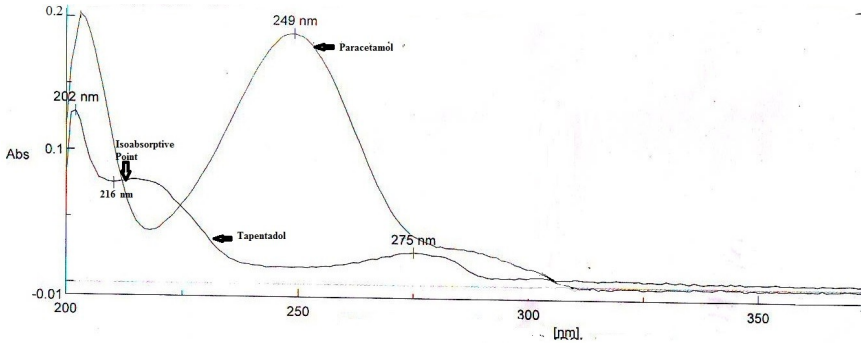


Figure 4 : Chromatogram obtained by using Phosphate buffer pH 3.0

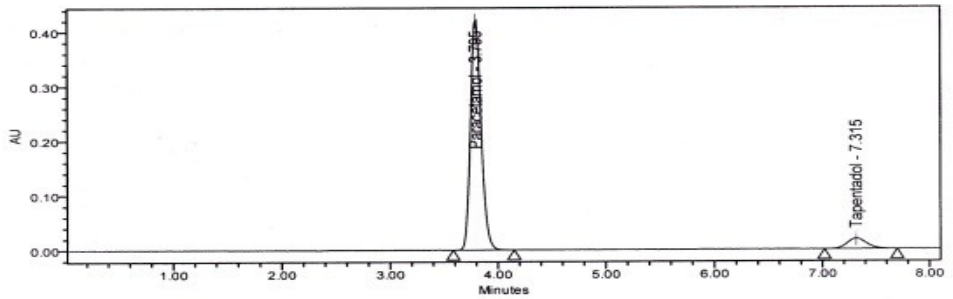


Figure 5 : Chromatogram for System Suitability

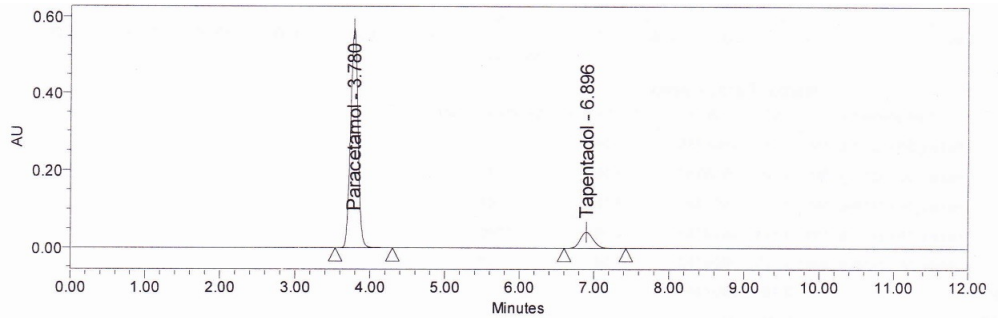


Figure 6: Chromatogram obtained for Laboratory mixture of TAP and PARA Showing retention time for TAP – 3.726 min. and PARA – 6.466 min.

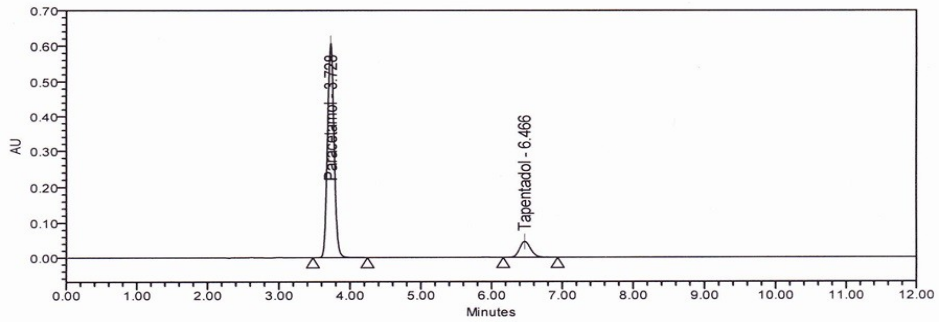


Figure 7 : Chromatogram obtained by Marketed Formulation of TAP and PARA showing retention time for TAP 6.593 min & PARA 3.754 min

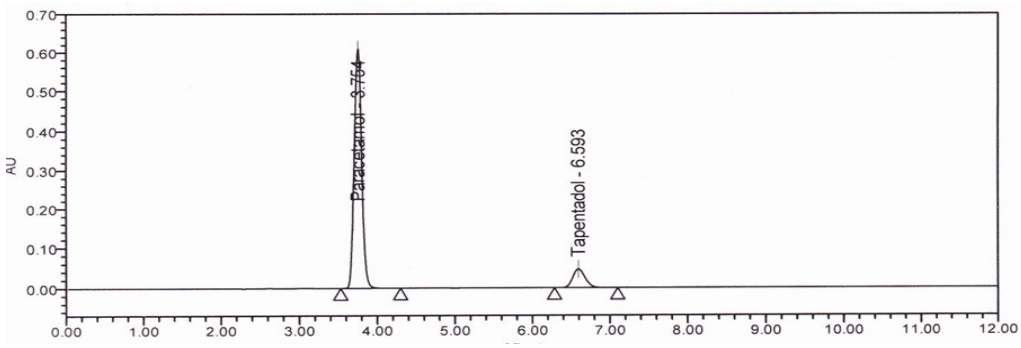


Figure 8 : Calibration Curve for TAP

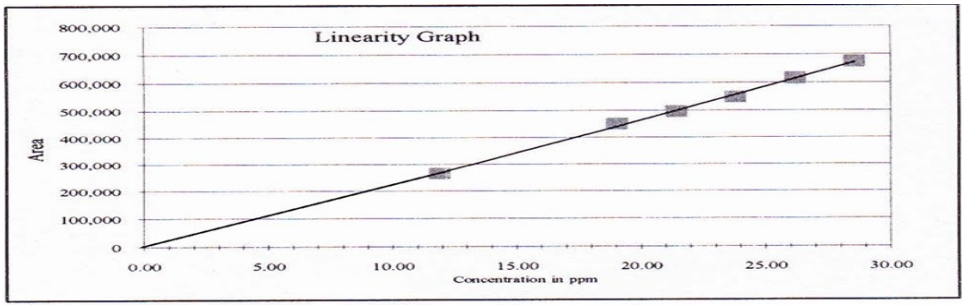


Figure 9 : Calibration Curve for PARA

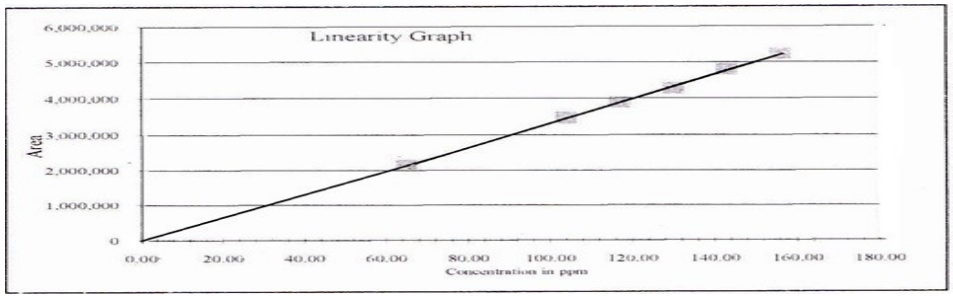


Figure 11: Chromatogram of Accuracy

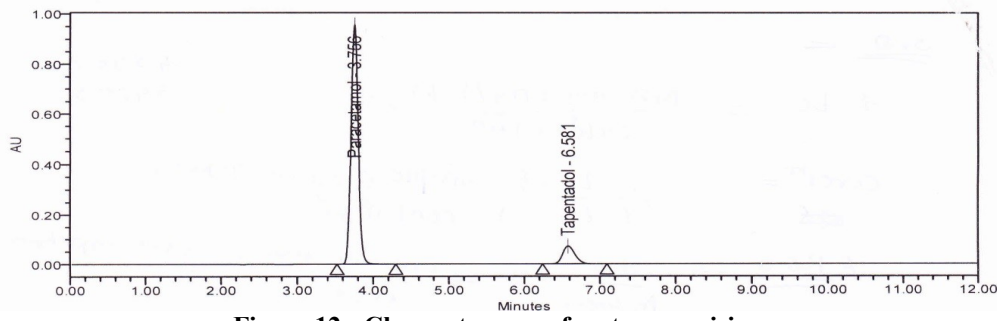


Figure 12 : Chromatogram of system precision

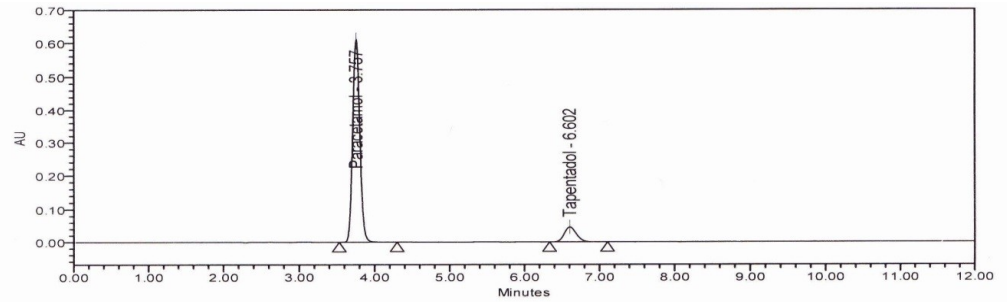


Figure 13 : Chromatogram of Intraday Precision

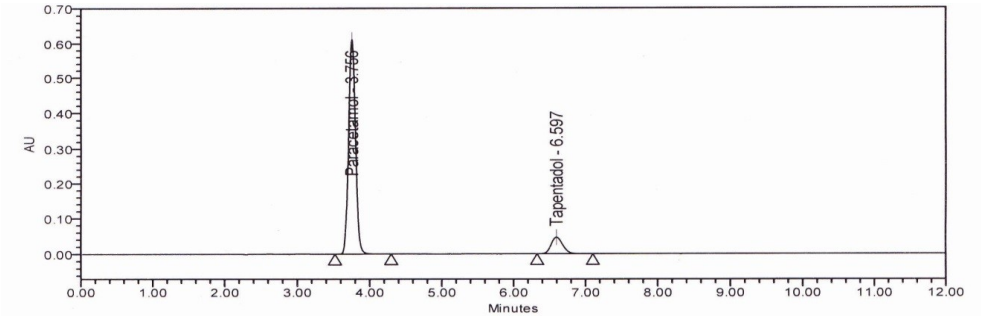


Figure 14 : Chromatogram of Ruggedness

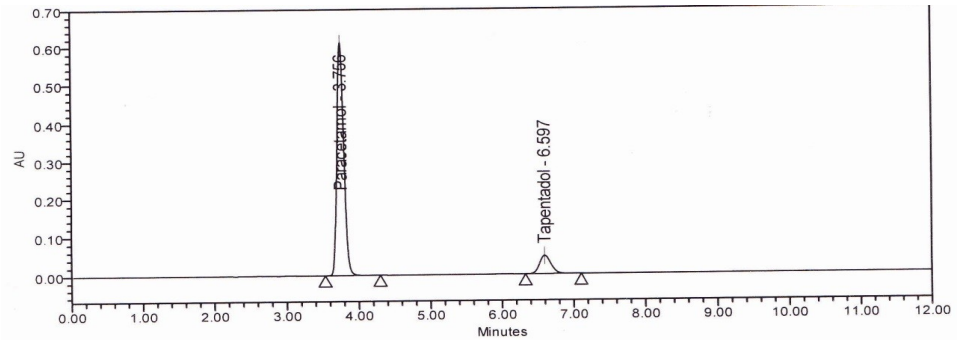


Figure 15 : Chromatogram of acid stressed degradation of drug product.

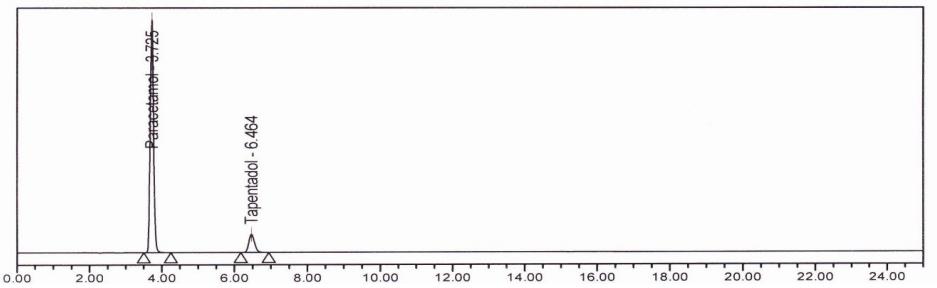


Figure 16 : Chromatogram of alkali stressed degradation of drug product

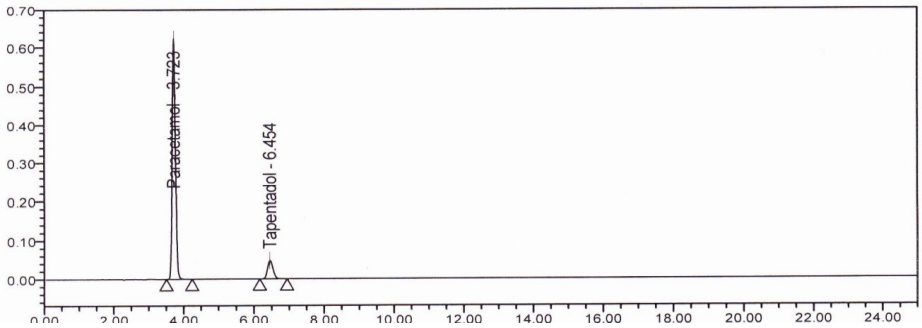


Table 1 : Forced Degradation Study

Sr. No	Degradation	Weight of sample	Stress condition
1.	Acid degradation	2594.60	1M HCl (water) 5 ml, Heat at 65°C for 60 Min
2.	Base degradation	2594.62	1M NaOH (water) 5 ml, Heat at 65°C for 60 Min
3.	Peroxide degradation	2594.65	3% H_2O_2 (water) 5 ml Heat at 65°C for 60 Min
4.	Photo degradation	2594.58	24 hours in UV chamber
5.	Thermal degradation	1807.5	50°C for 24 hours

Table 2 : Analysis of Physical Laboratory mixture for TAP and PARA

Sr. No.	Wt. of Std (mg)		Wt. of Sample		Peak area of Std.		Peak area of Sample		% Drug estimated	
	TAP	PARA	TAP	PARA	TAP	PARA	TAP	PARA	TAP	PARA
1	29.97	65.14	29.04	65.11	505709	398474	498523	395865	101.8	101.4
2	29.06	65.09	29.16	65.23	504844	397458	503265	386521	100.6	99.84
3	29.14	65.04	29.21	65.08	506228	395025	504563	394526	101.2	100.5
Mean									101.2	100.4
±SD									0.612	0.7830
% RSD									0.591	0.776

Table 3 : Analysis of Marketed Formulation

Std weight (mg)		Sample weight (mg)	Area of Std		Area of Sample		% Assay	
TAP	PARA		TAP	PARA	TAP	PARA	TAP	PARA
29.15	65.05	2594.12	522182	4024283	521938	402983	101.3	101.7
		2594.30			521655	402088	100.8	101.9
		2594.52			522192	402522	101.4	99.7
Mean							101.1	101.4
± S.D.							0.321	1.216
RS.D.							0.317	1.205

Table 4 : Results of Forced Degradation of Sample

Sr No	Amount in % w/w	Wt. Taken in mg	Area of TAP	Area of PARA	% LC of TAP	%LC of PARA	% degradation	% degradation
1	Control sample	2594.0	51981	40068	101.7	100.5	...	----
2	Acid degradation sample	2594.2	51103	29233	101.2	80.6	...	19.1
3	Base degradation sample	2594.1	39452	40895	85.2	100.1	16.5	----
4	Peroxide degradation sample	2594.2	51569	39256	100.8	99.4	---	----
5	Photo Degradation Sample	2594.2	51458	39856	100.3	99.7	...	----

4. Conclusion

The method provides selective quantification of TAP and PARA without interference from blank affirming its stability- indicating nature. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method was robust in the separation and quantification of TAP and PARA. This method can be used for the routine analysis of production samples. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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