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A Stability Indicating Method for the Simultaneous Estimation of Acetaminophen and Tramadol in Pharmaceutical Dosage Form

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ABSTRACT

A sensitive and selective RP-HPLC method is described for the determination of stability in Acetaminophen and Tramadol dosage forms. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of mixed Phosphate buffer pH: 3.4 Acetonitrile (30:70v/v/v), with detection of 236nm and flow rate at 1mL/min. Linearity was observed in the range 100-300 µg /ml for Acetaminophen (r^2 =0.994) & 10-30µg /ml for Tramadol (r^2 =0.994) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. No chromatographic interference from tablet excipients was found. The proposed methods were validated. The force degradation of the drugs was assessed by different environmental conditions. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Stability indicating, RPHPLC, Acetaminophen, Tramadol.

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INTRODUCTION

Acetaminophen is also known as paracetamol and chemically it is N-(4-hydroxyphenyl) acetamide, used as analgesics, non-narcotic**,** and antipyretics. The main mechanism proposed is the inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. Because of its selectivity for COX-2 it does not significantly inhibit the production of the pro-clotting thromboxanes[1-5].

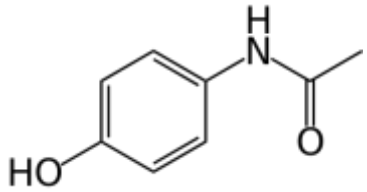

Figure 1 Molecular Structure of Acetaminophen

Tramadol is an opiate pain medication used to treat moderate to moderately severe pain. Chemically it is (1R, 2R)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexan-1-ol. It has two different mechanisms. First, it binds to the μ-opioid receptor. Second, it inhibits the reuptake of serotonin and norepinephrine[6-11].

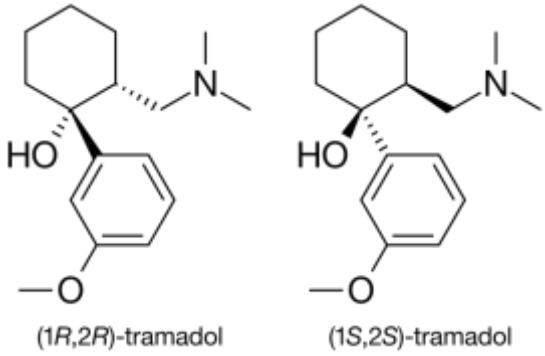

Figure 2 Molecular Structures of Tramadol

Methods such as HPLC, LC-MS, Protein precipitation, Capillary electrophoresis and Simultaneous UV-spectrophotometric methods are reported for estimation of Acetaminophen and Tramadol in combination. However, there were few methods reported for determination of stability of Acetaminophen and Tramadol. The focus of present study was to develop & validated a rapid, stable & economic HPLC method for the force degradation study in tablet dosage form.

MATERIALS AND METHOD

Chemicals and Reagents

Acetaminophen and Tramadol were gifted samples obtained from Chandra labs, Hyd. Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 μm

filtered water used for HPLC analysis and preparation of buffer. Buffers and all other chemicals were analytical grade.

Instrumentation

A Waters -1220 HPLC system consisting of a Waters pump - 2690, an inbuilt auto sampler, a column oven and Waters 2998 wavelength absorbance detector (PDA) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment. The mobile phase is a mixture of 30 volumes of mixed Phosphate Buffer pH 4.5: 70 volumes of Acetonitrile were prepared. with isocratic flow programming was used as mobile phase at 1.2 mL/min. The column was maintained at ambient temperature.

Chromatographic Conditions: The chromatographic elution was carried out in isocratic mode using a mobile phase is a mixture of 35 volumes of mixed Phosphate Buffer pH 4.5: 65 volumes of Acetonitrile. The analysis was performed at ambient temperature using a flow rate of 1 mL/min with a run time of 6 mins. The eluent was monitored using PDA detector. The mobile phase was filtered through 0.45 µm micron filter prior to use.

Preparation of standard stock solution of Acetaminophen

5020 +mg of Acetaminophen and was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.2ml to 10ml with methanol.

Preparation of standard stock solution of Tramadol

5mg of Tramadol was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 2ml to 10ml with methanol.

Preparation of mixed standard solution

Weigh accurately 50mg of Acetaminophen and 5mg of Tramadol in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the0 volume with mobile phase From above stock solution 200µg/ml of Acetaminophen and 20µg/ml of Tramadol is prepared by diluting 4ml of to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

10 tablets (each tablet contains 500mg of Acetaminophen and 50mg of Tramadol) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Acetaminophen (200µg/ml) and Tramadol (20µg/ml) were prepared by dissolving weight

equivalent to 50mg of Acetaminophen and 5mg of Tramadol and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 200µg/ml of Acetaminophen and 20µg/ml of Tramadol was made by adding 4ml of stock solution to 10 ml of mobile phase.

METHOD VALIDATION

The developed method was validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

Specificity Study

The specificity of the RP-HPLC method was checked by comparison of chromatograms obtained from standard, sample and the corresponding placebo.

Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 10-30 µg/mL for Tramadol and 100-300 µg/mL for Acetaminophen. The calibration curves were constructed by plotting peak areas versus concentration of Acetaminophen and Tramadol. The slope, Y-intercept and correlation coefficient were calculated.

Accuracy (% Recovery)

The accuracy of the method was evaluated in triplicate at three concentration levels, 50, 100 and 150 % of the target test concentration (15 µg/mL of Tramadol and 150 µg/mL of Acetaminophen). The percentages of recoveries were calculated.

Precision

Precision was investigated using the sample preparation procedure for six pure samples of Tramadol and Acetaminophen. Method Precision (Intra-day): The precision of the method was evaluated by carrying out six independent assays of 15 µg/mL of Tramadol and 150 µg/mL of Acetaminophen test samples against qualified reference standard. Six test samples were assayed against reference standard.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using signal - to - noise ratio of 3:1 and 10:1 as per ICH guidelines.

Robustness

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions: Flow rate (±0.2), column temperature (± 5°C) and wavelength of detection (± 2nm).

Ruguddeness:

The ruggedness of the method was evaluated by two different analyst with different instruments and lab.

System-Suitability Test

The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated using five replicate injections of the drugs at a concentration of 15 µg/mL of Tramadol and 150 µg/mL of Acetaminophen.

Forced Degradation or Stability Indicating Studies

Procedure

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, photolytic, and UV degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity

A) Acid degradation:

An accurately weighted quantity of tablet powder equivalent to about 20mg of Tramadol and 200mg of Acetaminophen was transferred to 100mL volumetric flask, sonicated with 50 mL of diluent with intermediate shaking for 15 min. To this flask 1mL of 0.1N HCl was added and this solution was placed in water bath at 60°C for 1 hr. Then the solution was allowed to cool at room temperature, and the sample solution was neutralized with 1mL of 0.1N NaOH. The volume was made up to the mark with diluent. 0.4mL of the above solution is diluted to 10 mL with diluent and the resulting solution was filtered. 10µL of the solution was injected and chromatograms were recorded for the same.

B) Alkali degradation:

An accurately weighted quantity of tablet powder equivalent to about 20mg of Tramadol and 200mg of Acetaminophen was transferred to 100 ml volumetric flask, sonicated with 50 mL of diluent with intermediate shaking for 15 min. To this flask 1mL of 0.1N NaOH was added and this solution was placed in water bath at 60°C for 1 hr. Then the solution was allowed to cool at room temperature, and the sample solution was neutralized with 1mL of 0.1N HCl. The volume was made up to the mark with diluent. 2mL of the above solution was diluted to 10 mL with diluent and the resulting solution was filtered. 10µL of the solution was injected and chromatograms were recorded for the same.

C) Thermal degradation:

The Drug substance was taken in Petri dish and exposed to a temperature of 105ºC for 1 hrs. Then the sample was taken and diluted with the diluent for further analysis. 10µL of the solution was injected and chromatograms were recorded for the same.

D) UV degradation:

The sample is kept under UV light for a period of minimum 24 hrs . The sample preparations were made with the diluents and 0.02 ml were injected and chromatogram was observed

E) Oxidation:

An accurately weighted quantity of tablet powder equivalent to about 20mg of Tramadol and 200mg of Acetaminophen was transferred to 100 ml volumetric flask, sonicated with 50 mL of diluent with intermediate shaking for 15 min. To this flask 1mL of 1.0% H_2O_2 was added and this solution was placed in water bath at 60°C for 1 hr. Then the solution was allowed to cool at room temperature. The volume was made up to the mark with diluent. 2mL of the above solution was diluted to 10 mL with diluent and the resulting solution was filtered. 10µL of the solution was injected and chromatograms were recorded for the same.

RESULTS AND DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Acetaminophen and Tramadol, different chromatographic conditions were applied & the results observed are presented. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution & the results observed are presented (Shown in Table 1 and 2). The results of Correlation coefficient (r) LOD, LOQ, Accuracy, Precision, Robustness and Ruggedness are shown in Table 1. The results of System Suitability Parameters consisting of Retention time, Theoretical plates, Asymmetry are shown in Table 2. The standard chromatogram of degradation studies of Tramadol and Acetaminophen in tablet dosage form are shown in figure 5. The calibration curve of Tramadol and Acetaminophen is shown in Figure 3 and 4 respectively.

Table 1: Results from Analysis and Calibration Curves

Parameters	Tramadol	Acetaminophen
Correlation coefficient (r)	0.994	0.995
LOD (µg/mL)	0.02	0.05
LOQ (µg/mL)	0.06	0.12
Accuracy (%) ± % RSD	99.28%	99.96%
Precision (% RSD)	0.76	1.33
Robustness	0.54	0.62
Ruggedness	0.80%	0.81%

Table 2: Results of System Suitability Parameters

Parameters	Tramadol	Acetaminophen
Retention time (min) ± % RSD	0.99 ± 0.05	0.95 ± 0.05
Theoretical plates ± % RSD	3572.63 ± 0.40	4534.51 ± 0.40
Asymmetry ± % RSD	1.25 ± 0.06	1.05 ± 0.06

Table 3: Results for Degradation studies of Acetaminophen

Conditions	Sample weight(mg)	Peak Area	% Claim	% Degradation
Sample Control	170	3476.752	100.20%	-
Alkali Degradation	71.23	3615.091	96.43	3.77%
Acid Degradation	68.954	3416.150	98.06%	2.14%
Thermal Degradation	69.59	3416.150	97.16	3.04%
Per Oxide Degradation	69.12	3170.146	92.41	7.79%
UV Degradation	67.56	3415.800	97.87	2.33%

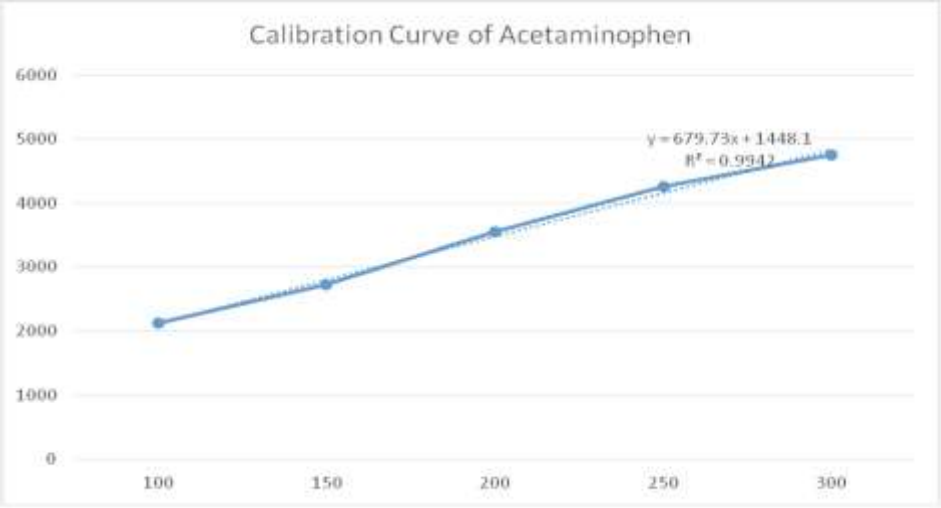

Figure 3 Calibration Curve of Acetaminophen

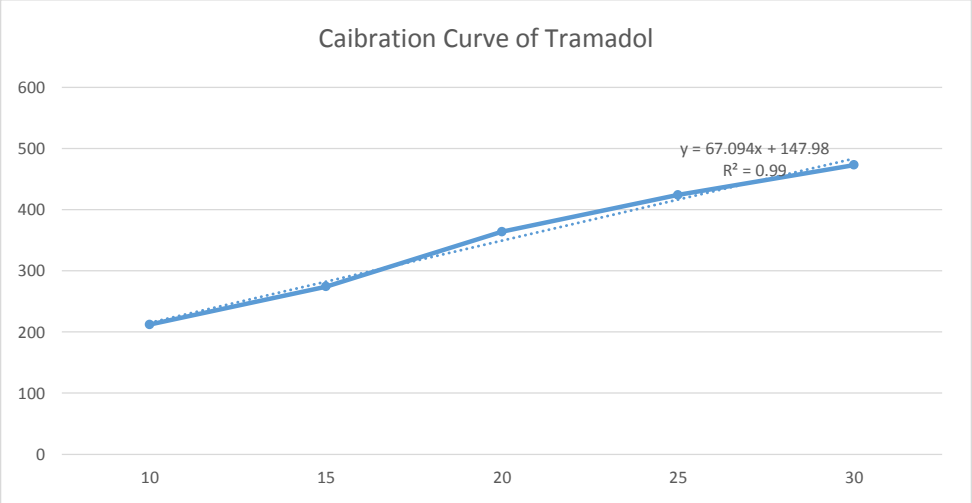

Figure 4 Calibration Curve of Tramadol

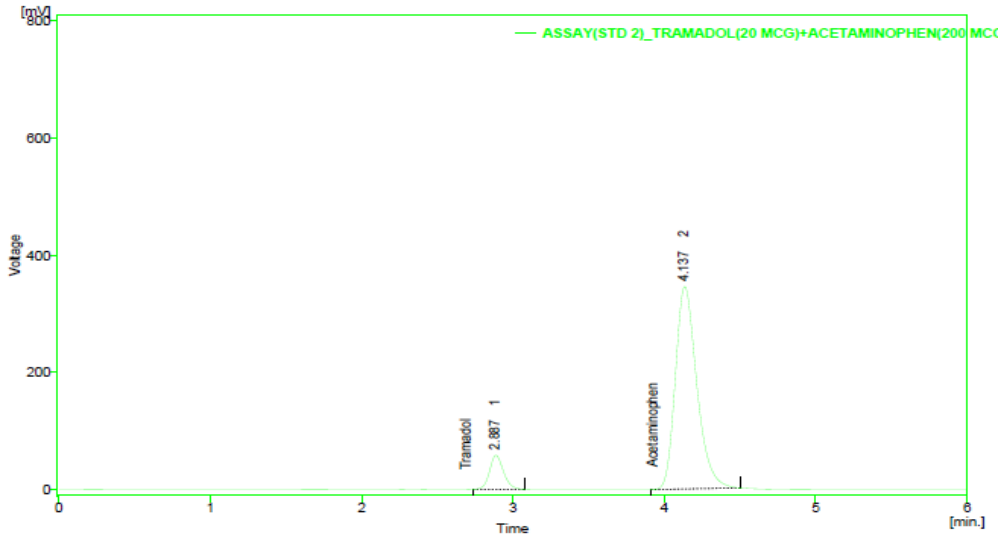

Figure 5: Assay of Acetaminophen and Tramadol

Table 4: Results for Degradation studies of Tramadol

Conditions	Sample weight(mg)	Peak Area	% Claim	% Degradation
Sample Control	68.96	367.796	98.36%	-
Alkali Degradation	67.59	337.970	93.55%	4.81%
Acid Degradation	69.89	346.724	92.82%	5.54%
Thermal Degradation	68.96	346.724	94.07%	4.29%
Per Oxide Degradation	70.56	376.883	94.94	3.42%
UV Degradation	68.954	347.830	94.38%	3.98%

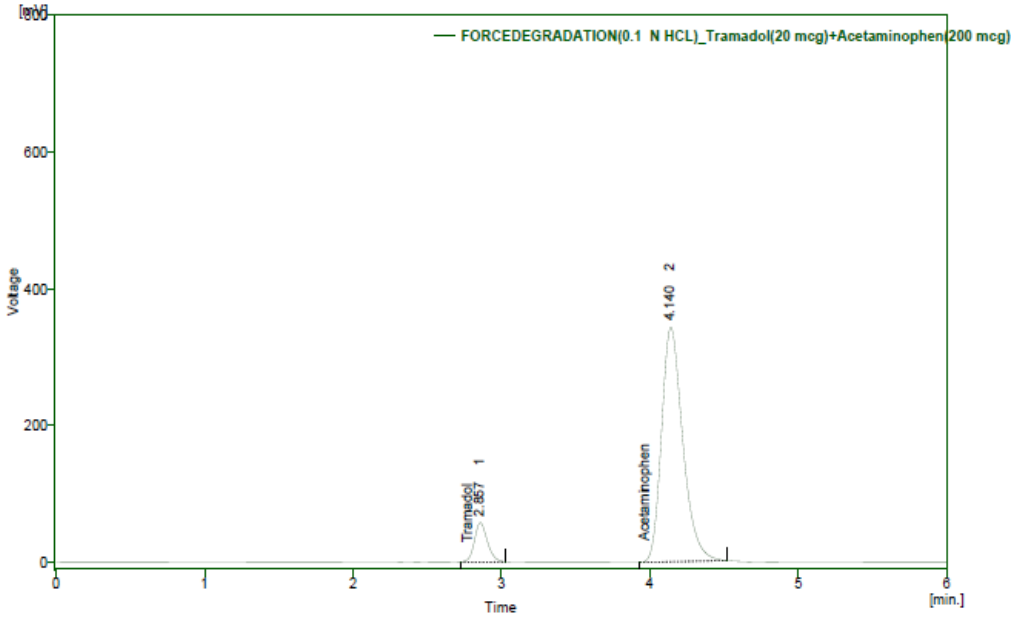

Figure 6 (a): Chromatogram for Acid Degraded Sample

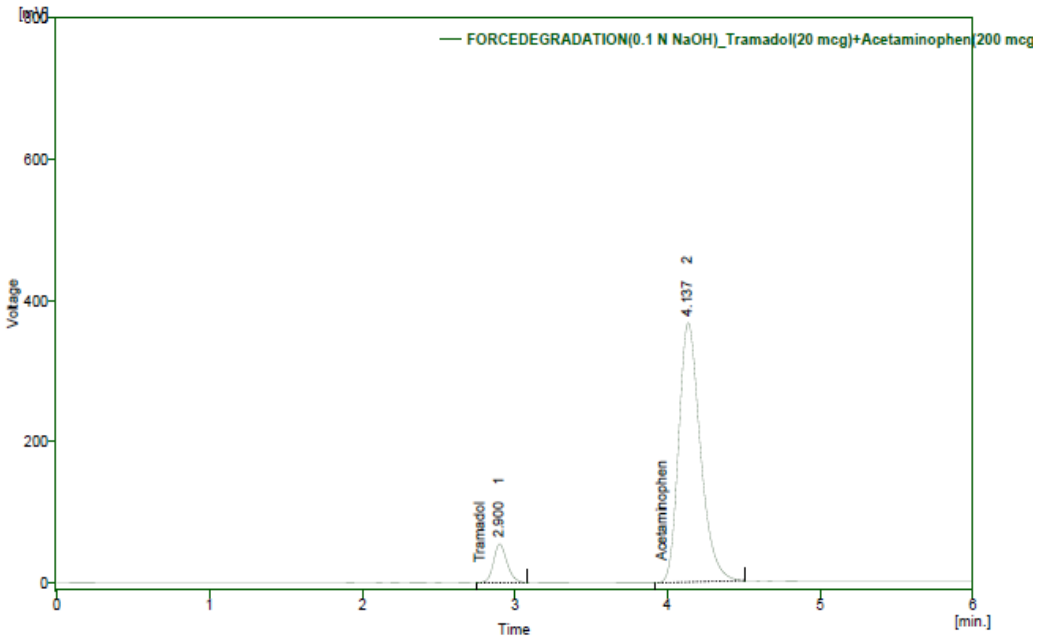

Figure 6 (b): Chromatogram for Alkali Degraded Sample

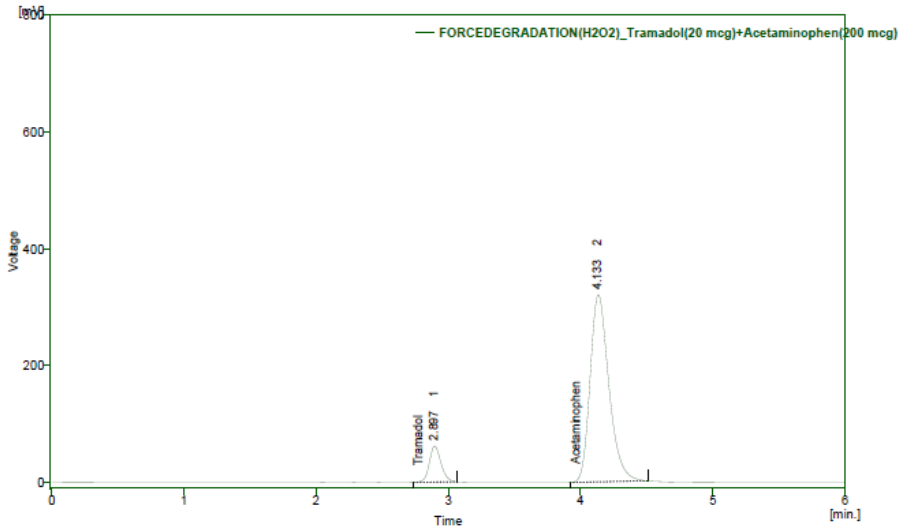

Figure 6 (c): Chromatogram for Peroxide Degraded Sample

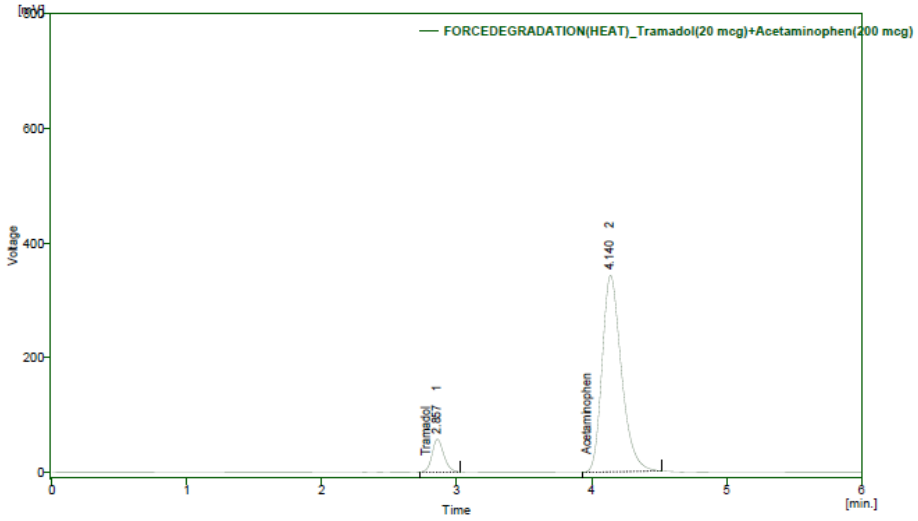

Figure 6 (d): Chromatogram for Thermal Degraded Sample

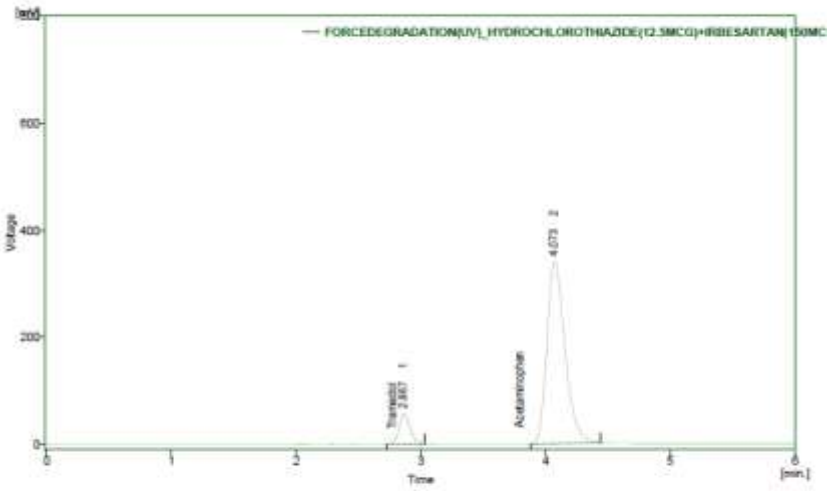

Figure 6 (e): Chromatogram for UV Degraded Sample

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Acetaminophen and Tramadol API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, impurity studies which can help in the analysis of Acetaminophen and Tramadol in different formulations

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