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RP-HPLC METHOD DEVELOPMENT FOR DEXKETOPROFEN TROMETAMOL AND PARACETAMOL IN PHARMACEUTICAL DOSAGE FORM AND ITS VALIDATION INCLUDING SHORT TERM STABILITY STUDY

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Keywords:

Dexketoprofen, Trometamol, Paracetamol, Stress testing, Degradation products, Stability indicating method, HPLC

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ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of Dexketoprofen trometamol and Paracetamol from tablet dosage form using a Hypersil BDS, C8 column (5µ, 4.6 mm x 250 mm) column and mobile phase composed of 0.05m sodium dihydrogen phosphate: acetonitrile: methanol (60: 20: 20 v/v) pH 5.5 adjusted with orthophosphoric acid, at flow rate of 1 ml/min. The retention time of Dexketoprofen Trometamol and Paracetamol were found to be 8.51 and 3.33 min respectively. Linearity was established for both drugs in the concentration range of 50-150 µg/ml. The percentage recoveries of Dexketoprofen Trometamol and Paracetamol were found to be in the range of 98.12%-101.82% and 98.15%-101.8% respectively. Detection was carried out at wavelength 256nm using photodiode array detector. Both the drugs were subjected to acid, alkali, neutral hydrolysis, oxidation, dry heat, and UV degradation. The degradation studies indicated Dexketoprofen trometamol and Paracetamol showed degradation in acid and oxidation. The degradation products of Dexketoprofen trometamol and Paracetamol in acidic and oxidation were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of Dexketoprofen trometamol and Paracetamol in tablet formulations.

INTRODUCTION

High performance liquid chromatography (HPLC) is one of the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability. High-performance liquid chromatography (HPLC) is a specific form of column chromatography generally used in analysis to separate, identify, and quantify the active compounds [1].

The basic need for new method development of drug analysis is that the drug or drug combination may not be available in official pharmacopeia or a proper analytical procedure may not be available in the literature due to patent regulations; analytical method for drug in combination with other may not be available. So it becomes necessary for routine work [2].

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH)1suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability-indicating and should be fully validated [3]. Dexketoprofen trometamol is chemically 2-Amino-2-(hydroxymethyl)-1,3-propanediol (S)-3- benzoyl-alpha-methylbenzeneacetate. It is a new, quick acting analgesic for the treatment of painful musculoskeletal conditions such as osteo-arthritis and low back pain. Its cyclo-oxygenase inhibitory effects decrease arachidonic acid metabolism to PGE1, PGE2, PGF1, PGF2, and thromboxanes A2 and B2, which accounts in part for its analgesic effects [4]. The structure of Dexketoprofen trometamol is shown in following Fig No -1.

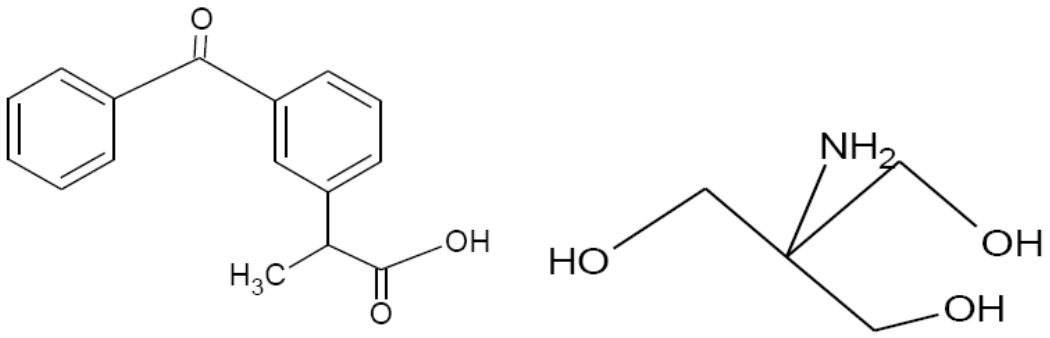


Fig No -1: Structure of Dexketoprofen trometamol

Paracetamol is Chemically N-(4-hydroxyphenyl) acetamide. The main mechanism of action of paracetamol is the inhibition of cyclooxygenase (COX), recent findings suggest that it is highly selective for COX-2 while it has analgesic and antipyretic properties comparable to those of aspirin or other NSAIDs. It is commonly used for the relief of headaches, other minor aches and pain. In combination with opioid analgesic, it can also be used in the management of more severe pain such as post surgical pain and providing palliative care in advanced cancer patients[5].The structure of Paracetamol is as shown in following Fig No -2.

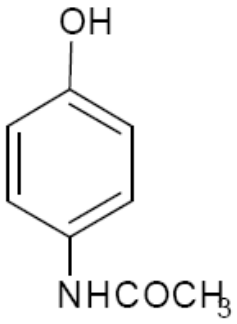


Fig No -2: Structure of Paracetamol

Literature survey reveals [6] that many analytical methods have been reported for determination of Dexketoprofen trometamol and Paracetamol individually. No single method was reported for the estimation in combined dosage form. The present work describes the development of a stability indicating RP-HPLC method, which can quantify these components simultaneously from a combined dosage form and also separate this component from its degradation products. The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [7],[8]. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine Dexketoprofen trometamol and Paracetamol simultaneously, in presence of its degradation products is rather a challenge for pharmaceutical analyst. Therefore, it was thought to study the stability of Dexketoprofen trometamol and Paracetamol under acidic, alkaline, oxidative, UV and dry heat conditions. This paper reports validated stability-indicating HPLC method for simultaneous determination of Dexketoprofen trometamol and Paracetamol in presence of their degradation products. The

proposed method is simple, accurate, reproducible, stability indicating and suitable for routine determination of Dexketoprofen trometamol and Paracetamol in combined dosage form. The method was validated in compliance with ICH guidelines.

MATERIALS AND METHODS

Sample, Reagents and Chemicals:

Active pharmaceutical ingredient (API) working standards and Test sample (Tablet with composition 25 mg Dexketoprofen trometamol and 325 mg Paracetamol) of Dexketoprofen trometamol and Paracetamol were received from Emcure Pharmaceuticals, Pune.

Instrumentation:

HPLC system (water) consisting of quaternary gradient pump, auto sampler, column oven, and PDA detector was employed for analysis. Chromatographic data was acquired using Empower software. The column used was Hypersil BDS, C8 column (5µ,4.6 mm x 250 mm).

Standard solution preparation:

Standard solution was prepared separately by transferring 75 mg and 32.5 mg of Dexketoprofen trometamol and Paracetamol respectively in a 100 ml diluent in volumetric flask. A 20 ml of diluents were added, sonicated and remaining volume was made up to the mark with diluents.

Diluents Preparation:

Diluents were prepared by 0.05M of Sodium Dihydrogen Phosphate: acetonitrile: methanol (60:20:20 v/v/v) and 5.5 pH was adjusted with orthophosphoric acid.

Method Development:

In method development, on the basis of trial and error method[9] , the mobile phase containing sodium dihydrogen phosphate: acetonitrile: methanol (60: 20: 20 v/v) was selected for separation of given drug. Final concentration 81.25 ppm containing standard Paracetamol and 6.25 ppm Dexketoprofen trometamol in combination was selected for method development.

HPLC conditions:

A mixture of acetonitrile, methanol and 0.05 M sodium dihydrogen phosphate buffer (pH adjusted to 5.5 with using orthophosporic acid) in the ratio of 20:20:60v/v was used as mobile phase and was filtered before use through 0.45 µm membrane filter. A constant flow of 1.0 ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 256 nm. The separation was carried out at 40°C temperature.

Analysis of formulation:

Weighed 20 tablets and calculated average weight. Weigh equivalent to 325 mg of Paracetamol was taken in 200 ml Volumetric Flask. Sonicated it for 10 min in Sonicator. Made up the volume with diluent. From these solution 5 ml was diluted to 100 ml volumetric flask. Made up the volume with diluents. The tablet sample solution was injected and chromatogram was obtained.

***Forced Degradation studies*: [7] [8]**

Drug product containing Dexketoprofen trometamol and Paracetamol was exposed under different conditions recommended by International Conference on Harmonization.

Stress Degradation by hydrolysis under acidic condition:

The drug product was added to 10 ml of 02.M HCL in 200 ml of volumetric flask. This solution was heated at 800C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was diluted upto 70 ml with diluents, sonicated for 10 min and made up the volume upto the mark with diluents. From these solution 5 ml was diluted to 100 ml volumetric flask.

Stress Degradation by Hydrolysis under Alkaline condition:

The drug product was added to 10 ml of 0.2M sodium hydroxide in 200 ml of volumetric flask. This solution was heated at 800C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was diluted upto 70 ml with diluents, sonicated for 10 min and made up the volume upto the mark with diluents. From these solution 5 ml was diluted to 100 ml volumetric flask. HPLC analysis was carried out using optimized conditions.

Stress Degradation by Hydrolysis under Neutral conditions:

The drug product was added to 10 ml of water in 200 ml of volumetric flask. This solution was heated at 800C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was diluted upto 70 ml with diluents, sonicated for 10 min and made up the volume upto the mark with diluents. From these solution 5 ml was diluted to 100 ml volumetric flask. HPLC analysis was carried out using optimized conditions.

Stress Degradation by Oxidation Studies:

The drug product was added to 10 ml of (30%) Hydrogen Peroxide in 200 ml of volumetric flask. This solution was heated at 800C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was diluted upto 70 ml with diluent, sonicated for 10 min and made up the volume upto the mark with diluents. From these solution 5 ml was diluted to 100 ml volumetric flask. HPLC analysis was carried out using optimized conditions.

Stress degradation by Dry heat Degradation:

The tablet powder (100 mg) was spread in petri dish and kept in oven at 1050C for 48 hr for dry heat degradation**.** The drug product was added in 200 ml of volumetric flask. The solution was diluted upto 70 ml with diluents, sonicated for 10 min and made up the volume upto the mark with diluents. From these solution 5 ml was diluted to 100 ml volumetric flask. HPLC analysis was carried out using optimized conditions.

Stress degradation by UV Degradation:

The tablet powder (100 mg) was spread in petri dish and exposed for 48 hr in UV light**.** The drug product was added in 200 ml of volumetric flask. The solution was diluted upto 70 ml with diluents, sonicated for 10 min and made up the volume upto the mark with diluents.

Method Validation:

The validation study was carried out [10] by International Conference on Harmonization.

Linearity and Range: For linearity study stock solution was prepared by adding 24 mg Dexketoprofen trometamol and 203 mg Paracetamol in 100 ml volumetric flask. Made volume with diluents. Peak area in conc. range 50-150 µg/ml was measured by HPLC at 256 nm. Calibration curve was plotted using mean concentration v/s area under curve (AUC).

Precision: Precision studies were performed by preparing the standards containing 81.25 ppm Paracetamol and 6.25 ppm Dexketoprofen trometamol six times and measuring the area under curve of drugs at 256 nm. For precision study tablet equivalent to 325 mg was taken in 200 ml volumetric flask. From these 5 ml was transferred to 100 ml volumetric Flask. Intraday and Inter day precision precision was performed by standard six times and measuring the area under curve of drugs at 256 nm at same day and at different day interval respectively.

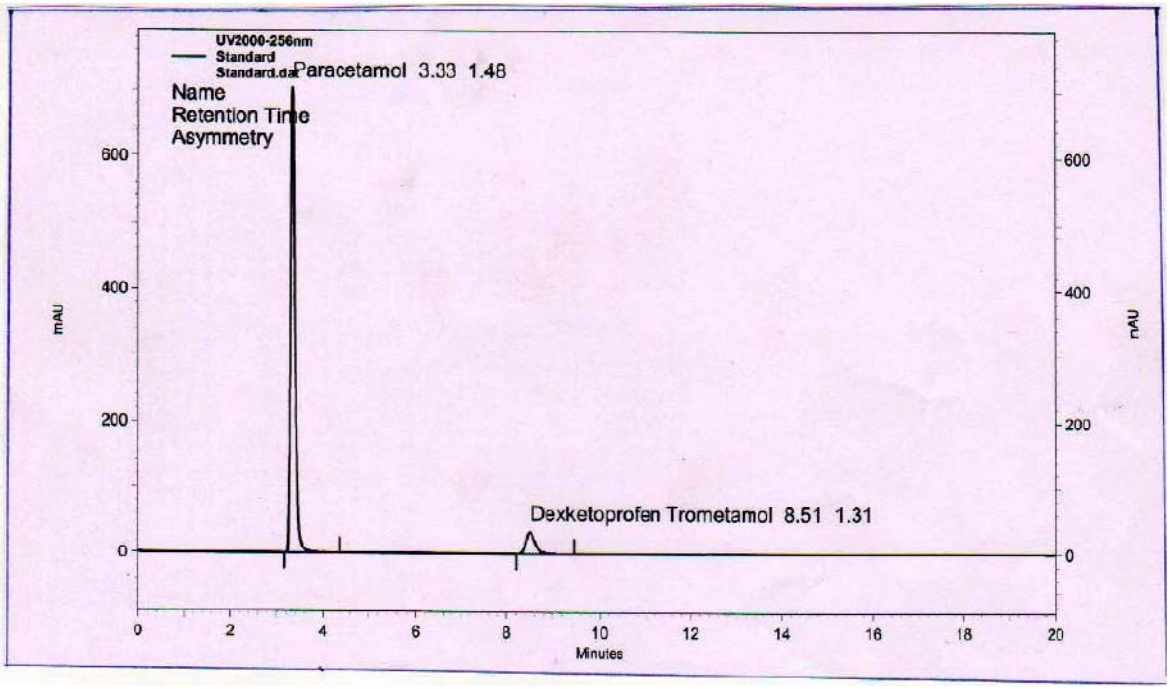
Accuracy: Accuracy of the method was carried out by adding known amount of each drug corresponding to five concentration levels 50%, 75%, 100% 125%, 125% of the label claim along with the excipients in triplicate.

Robustness: Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 0.9ml to 1.1ml. Composition of the mobile phase was changed by ± 5 % and the pH of mobile phase was changed from 5.5 to 5.3 and 5.7.

LOD and LOQ are calculated by using the values of slopes and intercepts of the calibration curves for both the drugs.

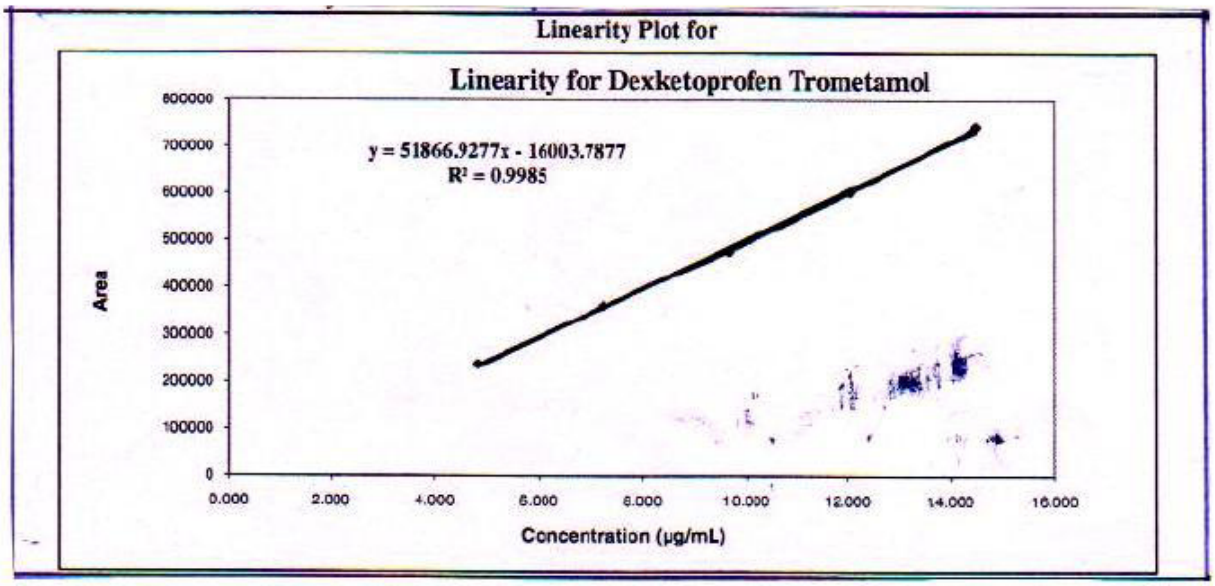
RESULT AND DISCUSSION

The mobile phase consisting of 0.05M sodium dihydrogen phosphate: acetonitrile: methanol (60:20:20v/v), pH 5.5 was adjusted with orthophosphoric acid; at 1 ml/min flow rate was optimized which gives two sharp well-resolved peaks with minimum tailing factor for Dexketoprofen trometamol and Paracetamol. The retention times for Dexketoprofen trometamol and Paracetamol is 8.51 and 3.33 min respectively. The typical chromatogram of marketed formulation is shown in following Graph no-1.

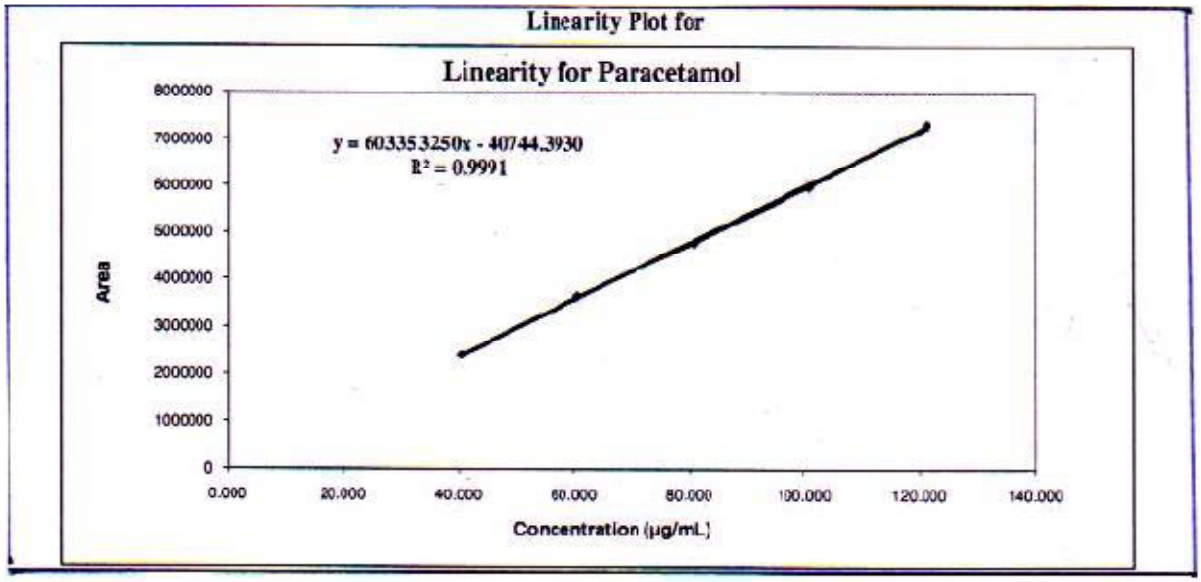


Graph no -1: Typical Chromatogram of Paracetamol and Dexketoprofen Trometamol.

UV overlain spectra of both Dexketoprofen Trometamol and Paracetamol showed that both drugs absorbed appreciably at 256 nm, so this wavelength was selected as the detection wavelength. The calibration curve for Dexketoprofen Trometamol and Paracetamol were found to be linear over the range of 50-150 µg/ml. The data obtained in the calibration experiments when subjected to linear-regression analysis showed a linear relationship between peak areas and concentrations in the range of 50-150 µg/ml for Dexketoprofen trometamol and Paracetamol. The equation of the regression line for Dexketoprofen trometamol is y = 51866x-1600.8 (r 2 = 0.9985) and for Paracetamol is y = 60335x-40744 (r 2 =0.9991). The result for both drugs is shown in following Graph no-8 and 9.



Graph no -8: Calibration curve for Dexketoprofen Trometamol.



Graph no -9: Calibration curve for Paracetamol.

The developed method was found to be precise as the % RSD values for intra-day and inter-day precision studies were found to be less than 2%. Good recoveries (98.15%-101.88% for Dexketoprofen trometamol and 98.12%-101.82% for Paracetamol) for both drugs were obtained at each added concentration, indicating that the method was accurate. Commonly used tablet excipients were subjected to chromatographic analysis and it was observed that there was no interfering peak at the retention time of Dexketoprofen trometamol and Paracetamol. Specificity

was also indicated by the resolution of Dexketoprofen trometamol and Paracetamol peak from the peaks of degradation product. The peak purity profile by PDA detector confirmed the specificity. During robustness check, the RSD (0.23-1.99%) and percentage of drug content area of injection (98.20-101.70%) is well within the acceptance criteria. The method was thus found to be robust since the monitored parameters i.e. flow rate, percent of organic content and pH of mobile phase were not significantly affected. The LOD of Dexketoprofen trometamol and Paracetamol was found to be 0.4 to 2.3 µg/ml and 0.01 to 0.15 µg/ml respectively.

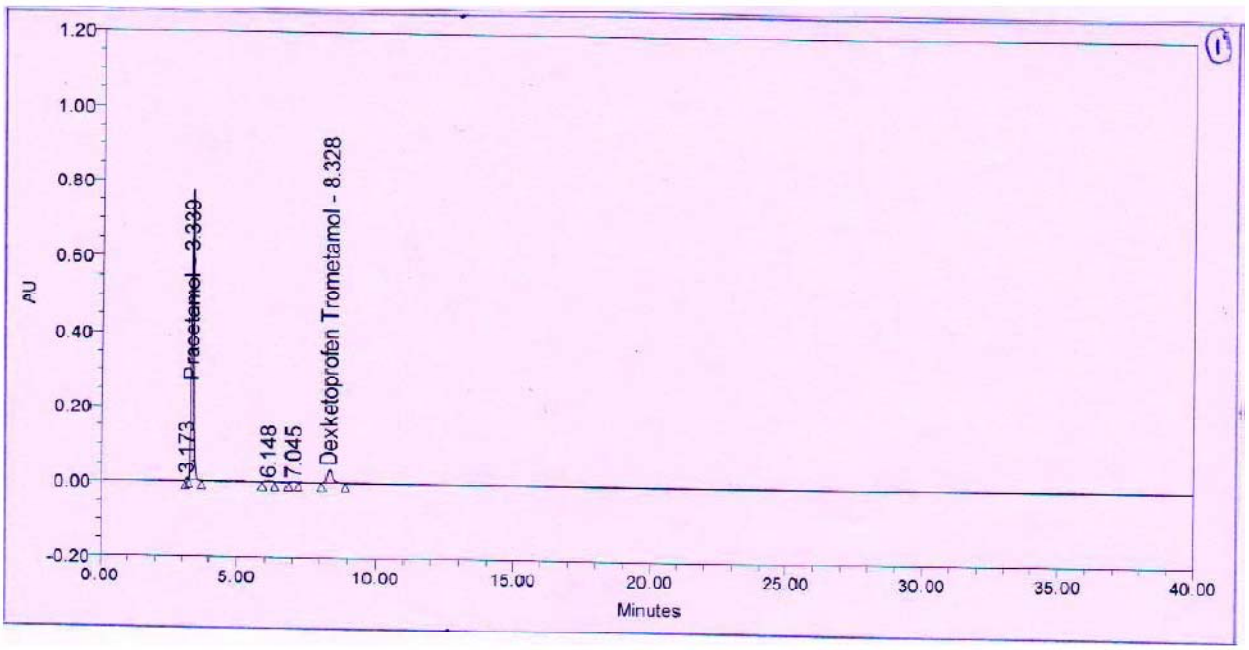
Table-2: Summary of validation parameters of proposed HPLC method

Parameter	Dexketoprofen trometamol		Paracetamol
Accuracy	99.15%-101%		99.50%-101%
Intermediate Precision	99.50%-101.50%		98%-101.20%
Method Precision	99.15%-101.20%		98.50-101%
Specificity	0.2		0.5
Linearity	0.9985		0.9991
Solution Stability	0.81-1.7		0.92-1.67
Robustness(% Assay)	+0.1ml	102.10	98.03
Flow Rate (± 0.1 ml)	-0.1ml	100.95	98.74
	+5%	101.93	98.94
Organic Content (± 5%)	-5%	101.99	99.14
	+0.2ml	102.32	98.90
PH (±0.2ml)	-0.2ml	102.35	99.65

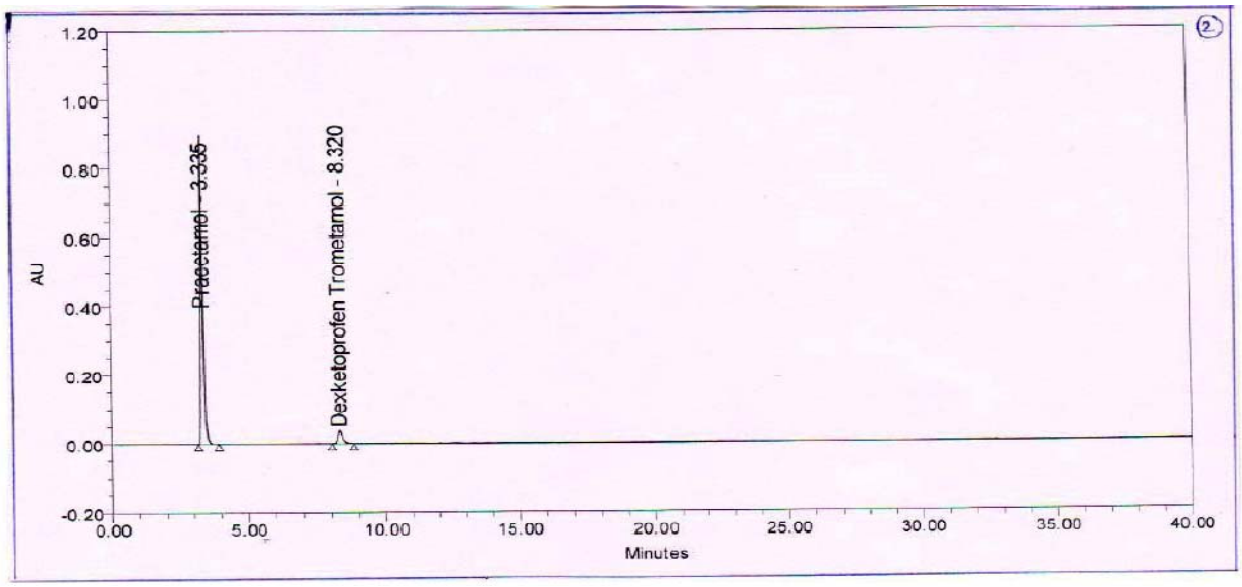
The HPLC chromatograms recorded for marketed formulation revealed no peaks within retention time around 10 minutes, and the peak purity was 99.99%. Graph no-1 and the peak purity index show that Dexketoprofen trometamol and Paracetamol is clearly separated from the response of any interfering peak(s) indicating good specificity of the proposed HPLC method. The values for intraday and interday variation are given in Table 2. In both cases, %RSD values were found well within 2% limit, indicating that the current method is repeatable. Low %RSD shows that the method has good precision. The % of RSD of robustness testing under different altered conditions is given in Table 2, indicating that the current method is robust.

Drug product containing Dexketoprofen trometamol and Paracetamol was found to degrade under acidic condition when drug product was treated with 0.2 M HCl. No additional degradation peaks were detected when drug product was treated with 0.1 M NaOH and 10 ml

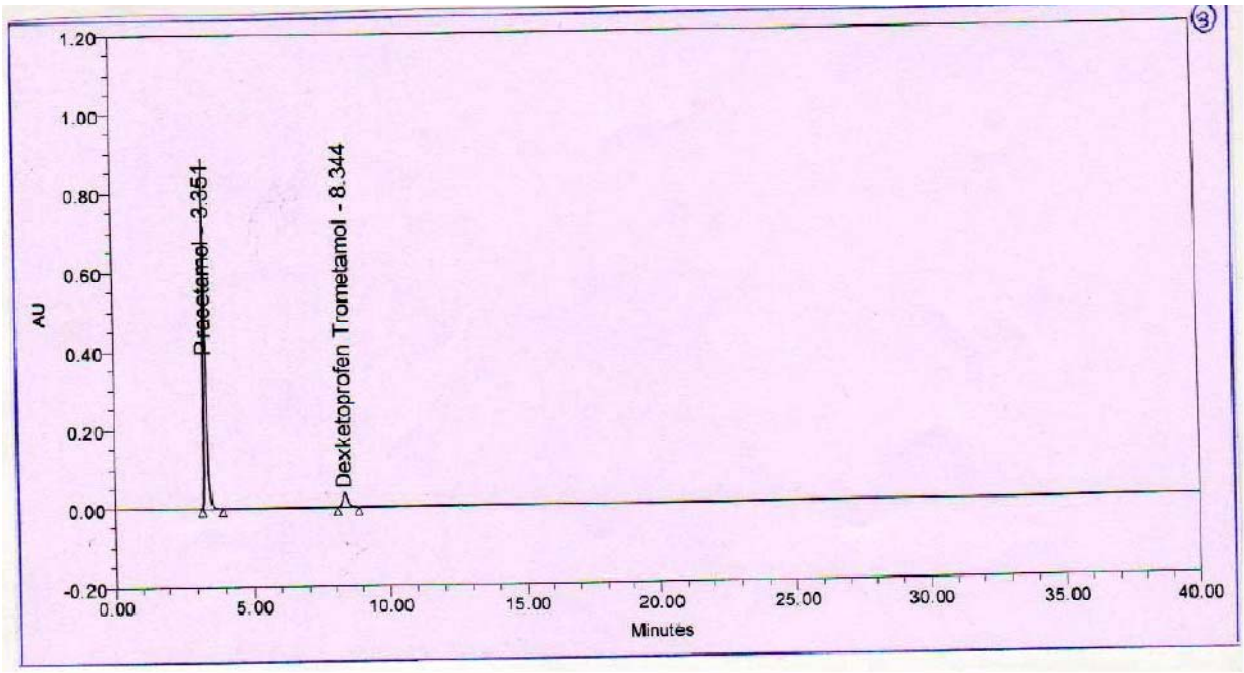
water. Two additional peaks are seen when drug product was treated with 30% hydrogen peroxide. No additional degradation peaks are detected when drug product was exposed under heat. When drug product was exposed to light source as per ICH guidelines, no additional peak is detected. The chromatogram of degradation studies of marketed formulation under acid, alkali. neutral, oxidation, thermal and UV are shown in following Graphs no - 2, 3, 4, 5, 6, 7 respectively.



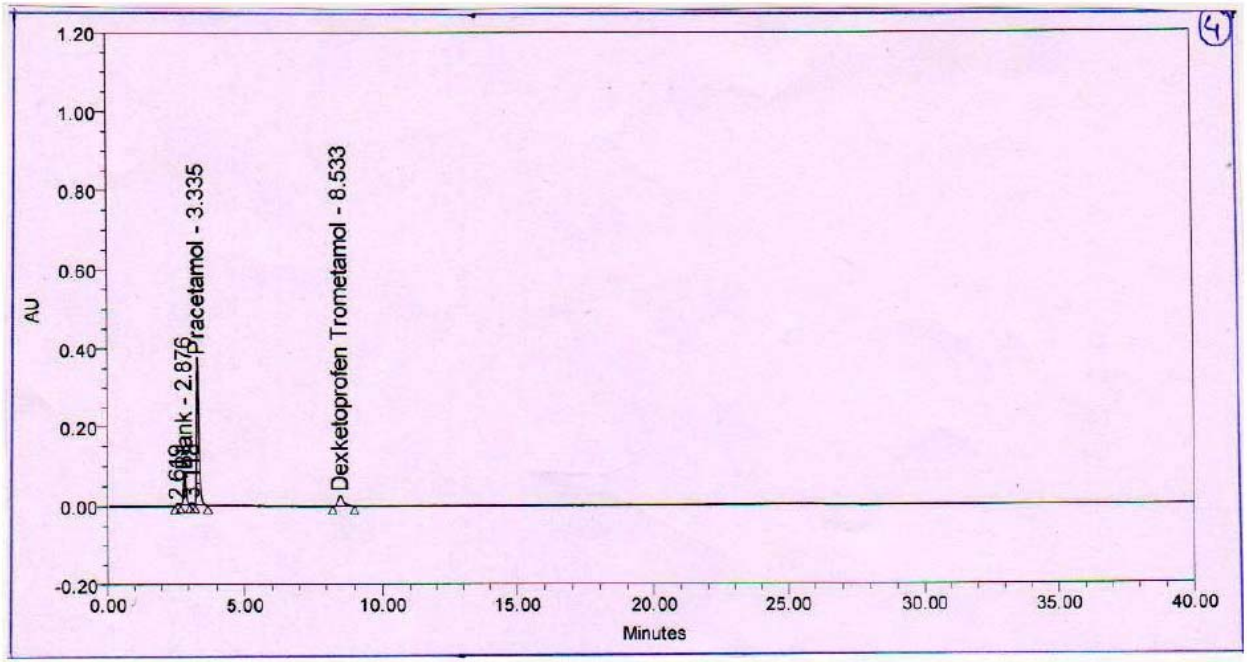
Graph no -2: Chromatogram of Marketed Formulation on Treatment with Acid (0.2M HCl)



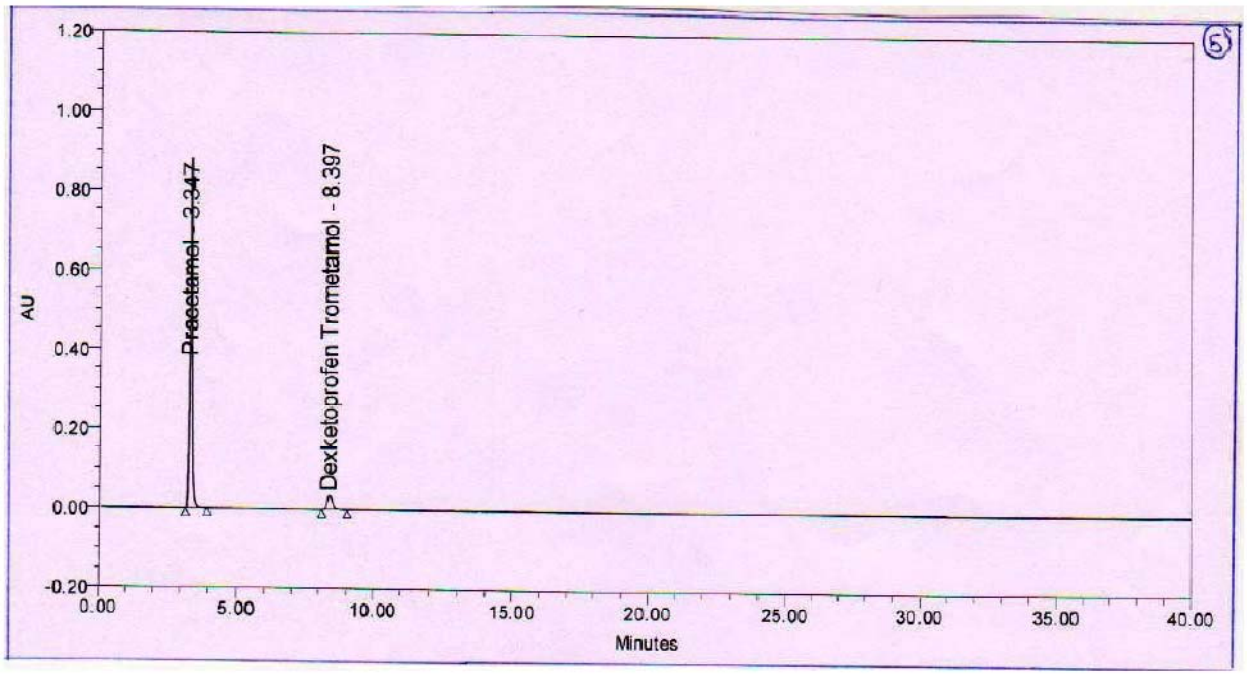
Graph no -3: Chromatogram of Marketed Formulation on Treatment with Alkali (0.2M NaOH)



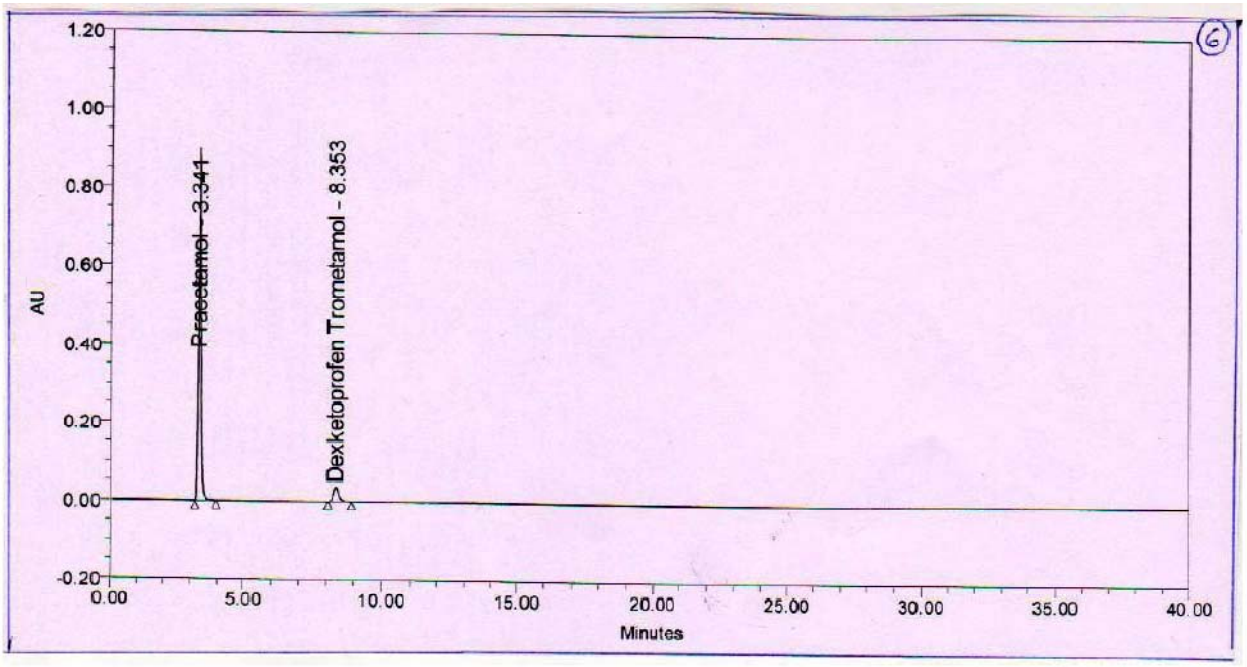
Graph no -4: Chromatogram of Marketed Formulation on Treatment with Water (Neutral)



Graph no -5: Chromatogram of Marketed Formulation on Treatment with H2O2 (30%)



Graph no -6: Chromatogram of Marketed Formulation on Thermal Degradation For 48 Hrs



Graph no -7: Chromatogram of UV-degradation of Marketed Formulation after 48 Hrs

All obtained results are shown in following Table no 1.

Table 1: Summary of Applied Condition of proposed HPLC method

Applied Condition	Degraded Amount(%)
Acid Condition	20.45
Alkaline Condition	No
Oxidative Condition	9.21
Neutral Hydrolysis	No
Thermal Degradation	No
UV Degradation	No

In stability testing significant degradation was obtained by 0.2m HCl and 30% hydrogen peroxide degradation. Chromatogram for 0.2M HCL degradation Graph no-2 exhibited two degradation products with retention times at 6.148 min and 7.045 min which were found to be well separated from each other and not affecting the Dexketoprofen trometamol and Paracetamol peaks purity. Chromatogram for H2O2 degradation, Graph no-5 exhibited a two well separated degradation product with retention time at 2.619 min and 2.876 min. All the degradation products obtained by stress conditions discussed above were found to be well separated from the principal peak which means that the Dexketoprofen trometamol and Paracetamol peaks were highly pure in all chromatograms obtained.

Thus the study shows that the drug product containing Dexketoprofen trometamol and Paracetamol undergoes degradation in acidic and oxidation conditions whereas it is relatively stable in alkaline, neutral and when exposed to dry heat and UV light. There is no acceptable difference between chromatogram of developed method and degradation studied products, as theoretical plates, retention time, asymmetry, % RSD, peak purity for both drug in standard and degradation peak study. A stability-indicating method was developed, which resolved all the degradation products formed under variety of conditions. The method proved to be simple, accurate, precise, specific and selective. Hence it may be used to assay of the product during stability studies [10].

CONCLUSION

It can be concluded that the method for Dexketoprofen trometamol and Paracetamol is linear, accurate, precise, robust, reproducible, economic, rapid and selective .The optimized HPLC conditions highlight chromatogram indicating two sharp, well-resolved peaks with minimum

tailing factor. The method is simple but it requires trial and error methods to get proper peak symmetry, resolution and time. Degradation study showed that these two active drugs are stable under conditions as alkali, neutral, thermal and UV light. The method the method gives a separate peak of degraded products from that of drugs .The developed method may be employed for analysis of stability samples of Dexketoprofen trometamol and Paracetamol. This method is found to be useful for simultaneous qualitative and quantitative analysis of Dexketoprofen trometamol and Paracetamol in tablet formulations.

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