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Stability Indicating RP-HPLC-PDA Method for Simultaneous Determination of Dexketoprofen Trometamol and Paracetamol from tablet dosage form

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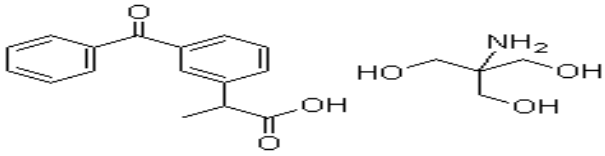
ABSTRACT

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, C_{18} column (5µ, 4.5 mm x 250 mm) column and mobile phase composed of 0.01m Potassium Dihydrogen Phosphate: acetonitrile (75:25 v/v) pH 6.0 adjusted with Triethylamine, at flow rate of 1 ml/min. The retention time of Dexketoprofen Trometamol and Paracetamol were found to be 6.732 and 3.256 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 254nm using photodiode array detector. The separation was carried out at 40^0 C temperature. 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 alkali. The degradation products of Dexketoprofen trometamol and Paracetamol in acidic and alkali 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.

Keywords: Dexketoprofen Trometamol, Paracetamol, stress testing, degradation products, stability indicating method, HPLC.

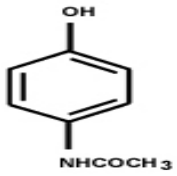
INTRODUCTION

Dexketoprofen trometamol is chemically 2-Amino-2-(hydroxymethyl)-1,3-propanediol (S)-3-benzoyl-alpha-methylbenzeneacetate. It is one of the most potent invitro inhibitors of prostaglandin synthesis. 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.



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 its peripheral anti-inflammatory activity is usually limited by several factors such as high level of peroxides present in inflammatory lesions[1]



Structure of Paracetamol

Dexketoprofen trometamol and Paracetamol alone can be estimated by various methods reported in the literature such as liquid chromatography (LC-DAD) [6], UV spectrophotometry[7,9&10] and HPTLC Method[8].

Literature survey reveals that many analytical methods are 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-PDA method, which can quantify these components simultaneously from a combined dosage form and also separate these 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[2&3]. 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 500mg Paracetamol)of Dexketo and Para were received from Emcure Pharmaceuticals, Pune. HPLC grade chemicals were obtained from Rankem.

Instrumentation:
HPLC system (water 2695 LC) consisting of quaternary gradient pump, autosampler, column oven, and PDA detector (2996) was employed for analysis. Chromatographic data was acquired using Empower software. The column used was Hypersil BDS, C_{18} column (5µ, 4.5 mm x 250 mm).

HPLC conditions:
A mixture of acetonitrile and 0.01 M potassium dihydrogen phosphate buffer (pH adjusted to 6.0 with using triethylamine) in the ratio of 25:75 v/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 254 nm. The separation was carried out at 40°c temperature.

Standard solution preparation:
Standard solution prepared separately by transferring 38 mg and 25 mg of Dexketo and Para respectively in a 100 ml and 50 ml volumetric flask. A 20 ml portion of diluent was added, sonicated and remaining volume was made up to the mark with diluent.

Diluent Peparation:
Diluent is prepared by 0.01M of Potassium Dihydrogen Phosphate: acetonitrile: methanol (75:20:5 v/v/v) and 6 PH adjusted with triethylamine.

Analysis of formulation:
Weigh 20 tablets and calculate average weight. Weigh equivalent to 50mg of Paracetamol in 100ml Volumetric Flask. Sonicate it for 10 min in Sonicator. Make up the volume with Diluent. Take 5 ml from these solution in 50 ml Volumetric flask and make up the volume with Diluent. The tablet sample solution was injected and chromatogram was obtained.[fig-1]

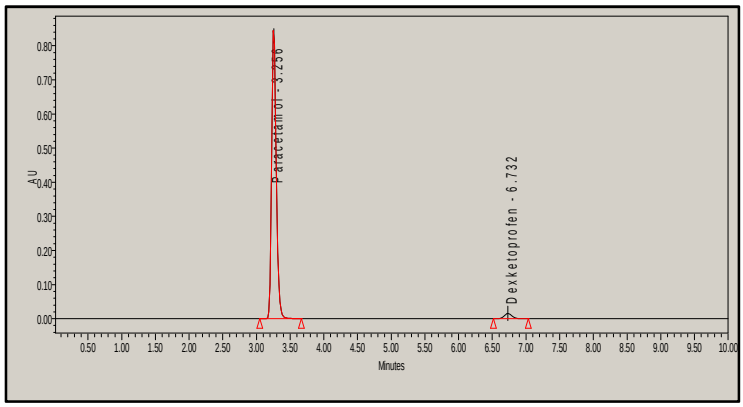


Fig -1: Typical Chromatogram of Paracetamol and Dexketoprofen trometamol

Method Validation:
The validation study was carried out by International Conference on Harmonization. the 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. Precision of the method was checked by analyzing the sampleson different times of the same day as well as on different days.

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.9 mL/min and 1.1 mL/min, composition of the mobile phase was changed by ± 5 % and The PH of mobile phase was changed from 6.0 to 5.8 and 6.2.

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

Forced Degradation studies:

Drug product containing dexketoprofen trometamol and paracetamol was exposed under different conditions recommended by International Conference on Harmonization.[2&3].

Stress degradation by hydrolysis under acidic conditions:

The drug product (39.94mg) was added to 10ml of 0.2M HCl in 100ml of volumetric flask. This solution was heated at 80 ^{0}C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was then adjusted to neutralize the solution with 0.1 M NaOH, then diluted upto 70 ml with diluents, sonicate for 10 mins and made up the volume upto mark with diluent. The chromatogram is shown in following [figure-2]

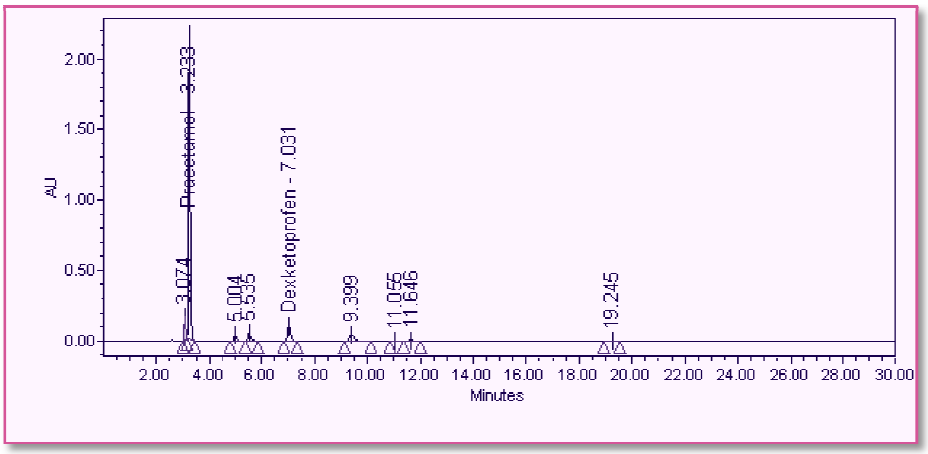


Fig-2: Chromatogram of Marketed Formulation on Treatment with Acid (0.2M HCl).

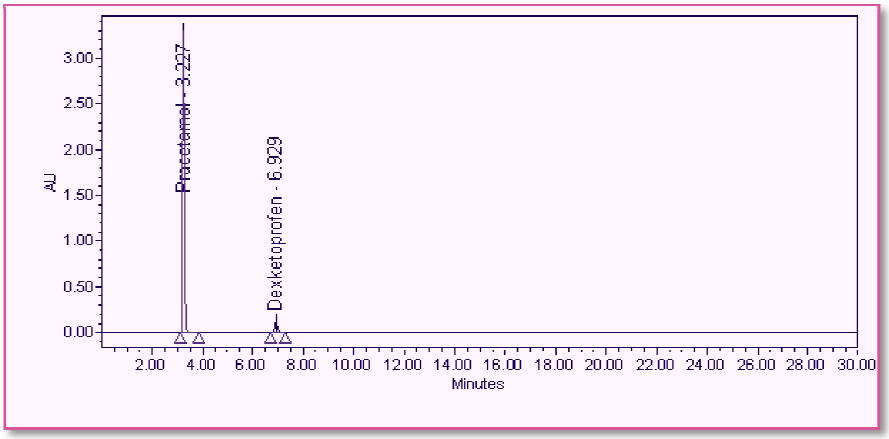


Fig -3: Chromatogram of Marketed Formulation on Treatment with Alkali (0.1M NaOH).

Stress degradation by hydrolysis under alkaline conditions:
The drug product (39.45 mg) was added to 10ml of 0.1M Sodium hydroxide in 100ml of volumetric flask. This solution was heated at 80 ^{0}C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was diluted upto 70 ml with diluent, sonicate for 10 mins and made up the volume upto mark with diluent.[fig-3]

Stress degradation by hydrolysis under neutral conditions:
The Drug Product (39.84 mg) was added to 10ml of water in 100ml of volumetric flask. This solution was heated at 80 ^{0}C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was diluted to 70 ml with diluent, sonicates for 10 mins and made up the volume upto mark with diluent.[fig-4]

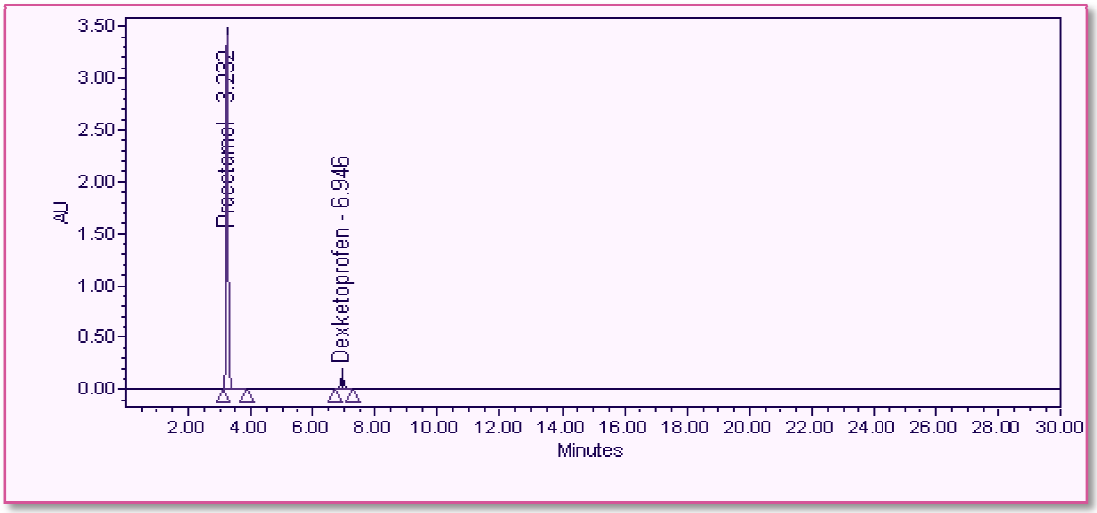


Fig-4: Chromatogram of Marketed Formulation on Treatment with water.

Stress degradation by Oxidation Studies:
The Drug Product (39.82 mg) was added to 10ml of (30%) hydrogen Peroxide in 100ml of volumetric flask. This solution was heated at 80 ^{0}C for 2 hr on a water bath then left to equilibrate at ambient temperature. The solution was diluted upto 70 ml with diluent, sonicate for 10 mins and made up the volume upto mark with diluent.[fig-5]

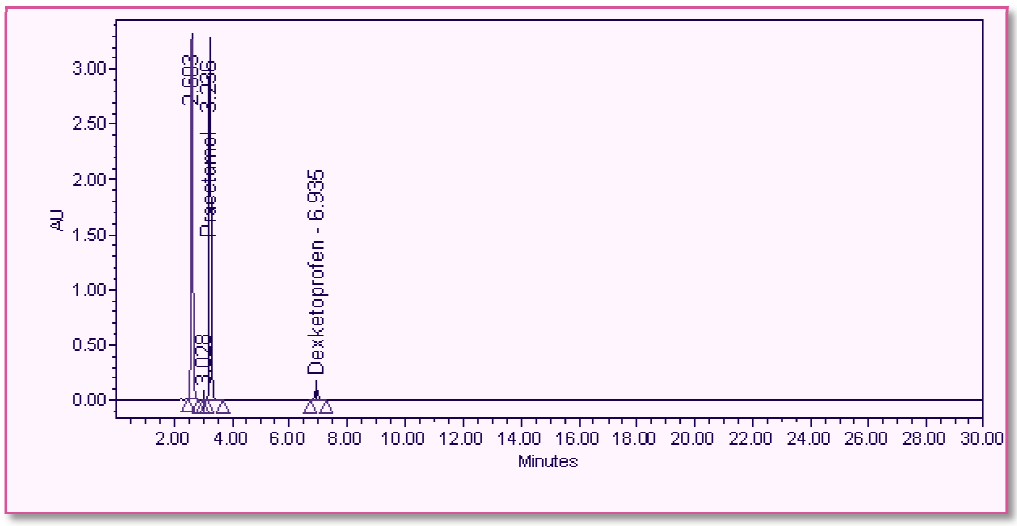


Fig-5: Chromatogram of Marketed Formulation on treatment with H_2O_2 (30%)

Stress degradation by Dry heat Degradation:
The tablet powder (100 mg) was spread in a Petri dish and kept in oven at 105^0C for 24 hrs for dry heat degradation. The drug Product (39.48 mg) was added to the 20ml of diluent in 100ml volumetric flask, sonicate for 10mins and made up the volume with diluents.[fig-6]

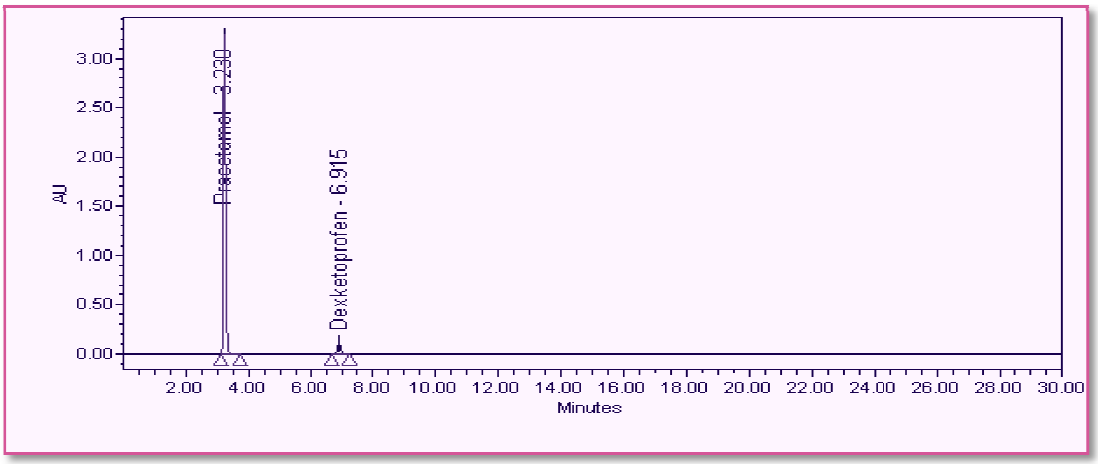


Fig-6: Chromatogram of UV-Degradation of Marketed Formulation after 24 Hrs UV Exposure.

Stress degradation by UV Degradation:
Tablet powder (100 mg) was spread in a Petri dish and exposed for 24 hrs in UV Light. The Drug Product (39.52 mg) was added to 20ml of diluent in 100ml volumetric flask, sonicate for 10mins and made up the volume with diluent.[fig-7]

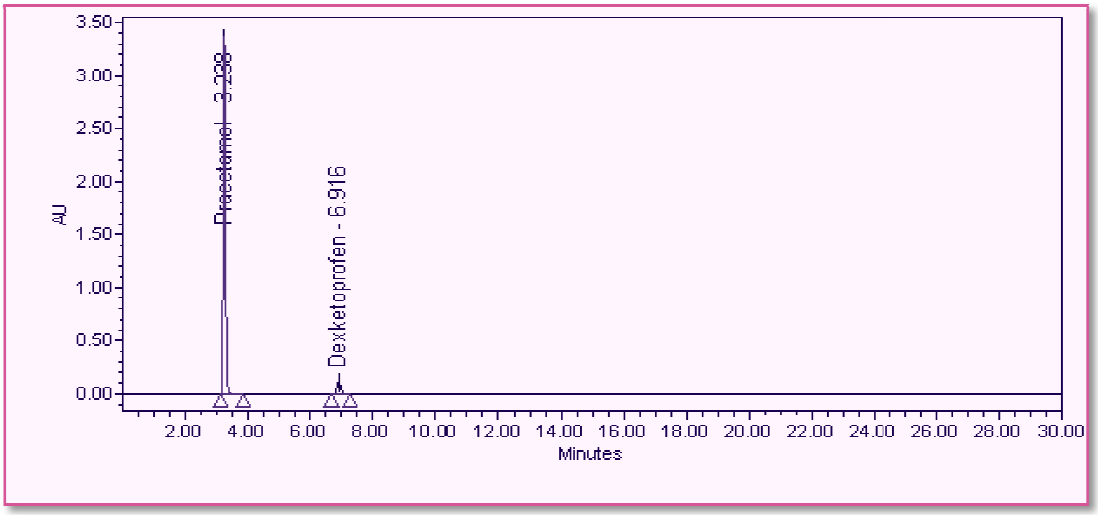


Fig -7: Chromatogram of Thermal Degradation of Marketed Formulation after 24 Hrs

RESULTS AND DISCUSSION

The mobile phase consisting of 0.01m Potassium Dihydrogen Phosphate: acetonitrile (75:25v/v) pH 6.0 adjusted with Trietylamine, at 1ml/min flow rate was optimised which given two sharp, well-resolved peaks with minimum tailing factor for Dexketoprofen trometamol and Paracetamol (fig.1). The retention times for Dexketoprofen Trometamol and Paracetamol were 6.732 and 3.256 min respectively. UV overlain spectra of both Dexketoprofen Trometamol and Paracetamol showed that both drugs absorbed appreciably at 254 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 = 70323x+5720.9 (r^2 = 0.9994) and for Paracetamol is y = 6983x+26407 (r^2 =0.9996). The result for both drugs is shown in following figure:

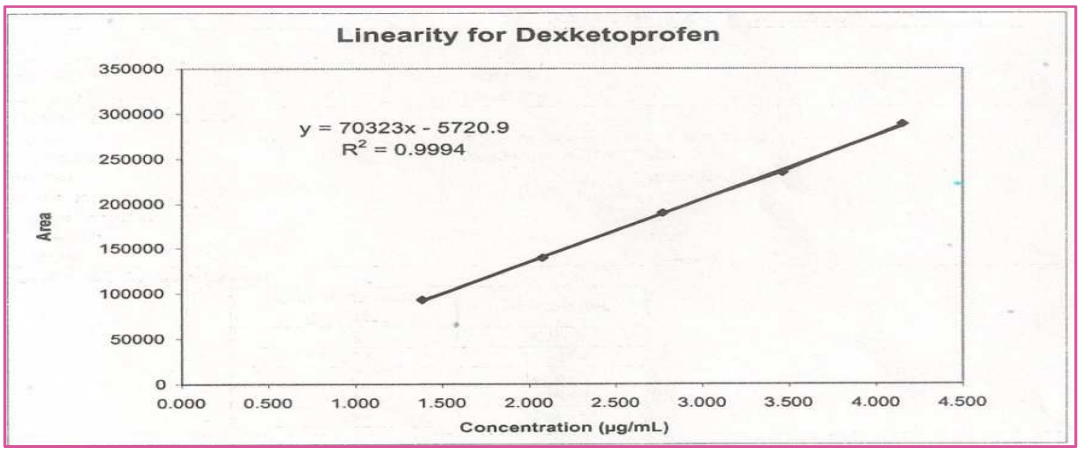


Fig No. 8: Calibration curve for Dexketoprofen Trometamol.

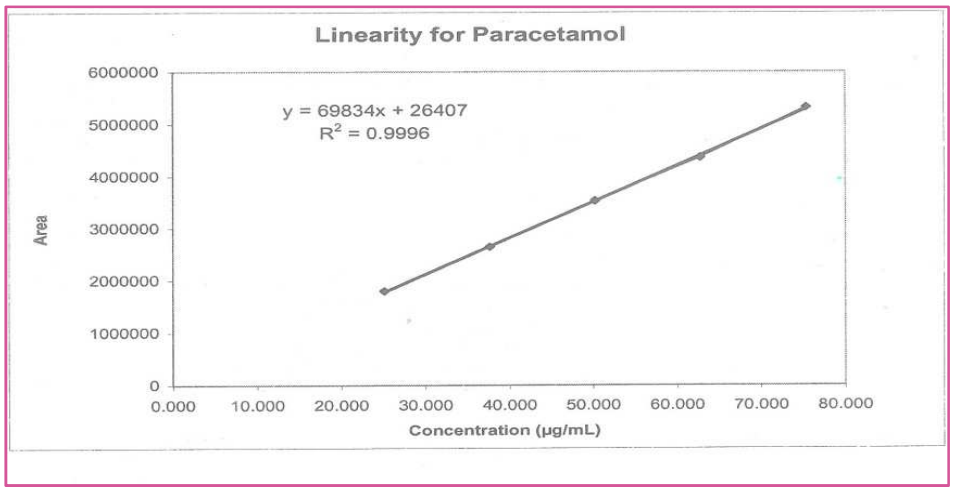


Fig No. 9: Calibration Curve for Paracetamol

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 0.0 1 to 0.15 µg/ml respectively. LOQ of

Dexketoprofen trometamol and Paracetamol was found to be 1.3 to 7.0 µg/ml and 0.05 to 0.4 µg/ml respectively. The values indicate that the method is sensitive Summary of validation parameters of proposed HPLC method is shown in following table:

Table-1: Summary of validation parameters of proposed HPLC method

Parameter	Dexketoprofen Trometamol		Paracetamol
Accuracy(% amount Recovered)	98.15%-101.88%		98.12%-101.82%
Intermediate Precision(% Assay)	99.74%-101.96%		99.19%-101.76%
Method Precision(% Assay)	98.12%-101.82%		98.07%-101.89%
Specificity(% RSD)	0.1		0.3
Linearity$^{(r2)}$	0.9996		0.9994
Solution Stability(% RSD)	0.63-1.5		0.84-1.45
Robustness(%Assay) 1. Flow Rate (± 0.1 mL)	+0.1mL	99.82%	101.5%
	-0.1mL	99.56%	101.3%
2. Organic Contents (± 5%)	+5%	98.25%	101.6%
	-5%	98.20%	101.7%
3. PH (±0.2mL)	+0.2mL	98.28%	101.4%
	-0.2mL	99.32%	100.3%

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 . No additional degradation peak was detected when drug product was treated with 10ml water. Two additional peaks were seen when drug product was treated with 30% Hydrogen peroxide. No additional degradation peak were detected when drug product was exposed under heat. When drug product was exposed to light source as per ICH guidelines, but no additional peak was detected. All obtained results were shown in following Table 2:

Table 2: Summary of Applied Condition of proposed HPLC method

Applied Condition	Degraded Amount(%)
Acid Condition	16.82%
Alkaline Condition	No
Oxidative Condition	1.7%
Neutral Hydrolysis	No
Thermal Degradation	No
UV Degradation	No

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. 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.

CONCLUSION

It can be concluded that the method separates the drugs from their degradation products; it may be employed for analysis of stability samples of Dexketoprofen Trometamol and Paracetamol. However characterization of degradation products was not carried out.

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