WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 7, 905-918. Research Article ISSN 2277– 7105

DEVELOPMENT OF FORCED DEGRADATION METHOD AND VALIDATION FOR THE ESTIMATION OF DEXRABEPRAZOLE IN MARKETED FORMULATION

Ashna Prasad*, Dr. Narendra Pratap Sengar, Mr. Manish Sharma***, Dr. Jitendra Banweer******

*Student, Department of Pharmaceutical Chemistry, Sagar Institute of Research & Technology Pharmacy, Bhopal (M.P.)

**Head department of Pharmaceutical Chemistry, Sagar Institute of Research & Technology Pharmacy, Bhopal (M.P.)

***Assistant Professor, Department of Pharmaceutical Chemistry, Sagar Institute of Research & Technology Pharmacy, Bhopal.

**** Dean & Director of SAGE University Bhopal (M.P.).

Article Received on
20 April 2022,
Revised on 10 May 2022,
Accepted on 31 May 2022
DOI: 10.20959/wjpr20227-24244

***Corresponding Author**
Ashna Prasad
Student, Department of Pharmaceutical Chemistry, Sagar Institute of Research & Technology Pharmacy, Bhopal (M.P.)

ABSTRACT

In the present research work, a successful attempt was made for “Development of forced degradation method and validation for the estimation of Dexrabeprazole in marketed formulation” which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in blood plasma. Liquid chromatographic system from waters comprising of manual injector, waters 515 pump for constant flow and constant pressure delivery and U.V. Vis. Detector connected to data ace software for controlling the instrumentation as well as processing the data generated were used. Drug sample was extracted by precipitating method using 5ml of methanol for each ml of plasma sample. The proposed methods were found to be linear with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent. The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the

value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

1. INTRODUCTION

1.1 Method Development1-4 Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency and performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physiochemical properties (pKa, logP, solubility) and determining which mode of detection would be suitable for analysis. The majority of the analytical development effort goes into validating a stability indicating HPLC method. The goal of the HPLC method is to try and separate quantify the main active drug, any reaction impurities, all available synthetic intermediates and degradants. Analysis can be divided in to two classes, i.e. Qualitative analysis and Quantitative analysis. Qualitative analysis gives an indication of the identity of the chemical species in the sample. Quantitative analysis estimates, how much quantity is present in a mixture. Modern analytical chemistry is functioning by instrumental analysis. Separation of components in a mixture is based on their interaction between a stationary and a mobile phase. These interaction differences are achieved based on the properties such as polarity, electric charge (for ionic compounds), pH, functional groups and size of the molecule5-6. Different types of quantitative analytical techniques are there, for eg: HPLC, Gas chromatography, TLC, Ion chromatography and Column chromatography, UV/Visible spectroscopy, FT-IR, LC-MS, GC-MS, MASS and NMR.

There are many steps involve in method development which are:

- Physicochemical properties of drug
- Set up HPLC conditions
- Sample preparation
- Method optimization
- Validation of developed method

2. EXPERIMENTAL WORK

2.1 Identification and Characterization of drug

2.1.1 Solubility: Solubility of drug was observed by dissolving them in different solvents according to IP.

Table 2.1.1: Solubility of drug in different solvents.

S. No.	Solvent	Dexrabeprazole
1.	Water Soluble	Soluble
2.	Ethanol Soluble	Soluble
3.	Acetonitrile Soluble	Soluble
4.	0.1 N HCl Freely soluble	Freely Soluble
5.	0.1 N NaOH Soluble	Soluble
6.	Chloroform Insoluble	Insoluble
7.	Methanol Soluble	Soluble

2.1.2 Identification of drugs by Melting point determination.

Melting point of drugs was determined by using Digital melting point apparatus.

Table 2.1.2: Melting Point of Drugs

Drug Name Melting Point Standard Value Dexrabeprazole 152-156°C 152°C.

2.2 RP- HPLC Method development of Dexrabeprazole

2.2.1 Selection of Mobile Phase

Initially to estimate Dexrabeprazole in fix dosage form number of mobile phase in different ratio were tried. A result was shown in Table 2.3. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 10Mm KH_2PO_4: Methanol in the ratio of 20:80 v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

2.2.2 Procedure for preparation of mobile phase

Preparation of Mobile phase Take 50 proportion of Acetonitrile and 50 proportions of methanol mix and filtered through 0.45 µ filter paper. Table 7.3: Mobile Phase Selection Mobile Phase Ratio Retention Time Remark Methanol: Water (50:50v/v) 50: 50 v/v Poor Resolution Not Suitable 10Mm KH_2PO_4: Methanol 20: 80 v/v Good Resolution Suitable Figure 7.1: Chromatogram of Dexrabeprazole in 10Mm KH_2PO_4: Methanol (20:80v/v).

2.2.3 Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents. Experimental Work Page 31 7.2.4.

Selection of separation variable: Table 7.4: Separation Variable Variable Condition Column Dimension 250mm x 4.60mm Particle Size 5µ Bonded Phase Octadecylsilane (C18) Mobile Phase 10Mm KH2PO4 20 Methanol 80 Diluents Methanol Flow rate 1.0 ml/min Temperature Ambient Sample Size 20 µl Detection wavelength 258mm Retention time Dexrabeprazole 4.021± 0.03min.

1. Preperation of Stock Solution

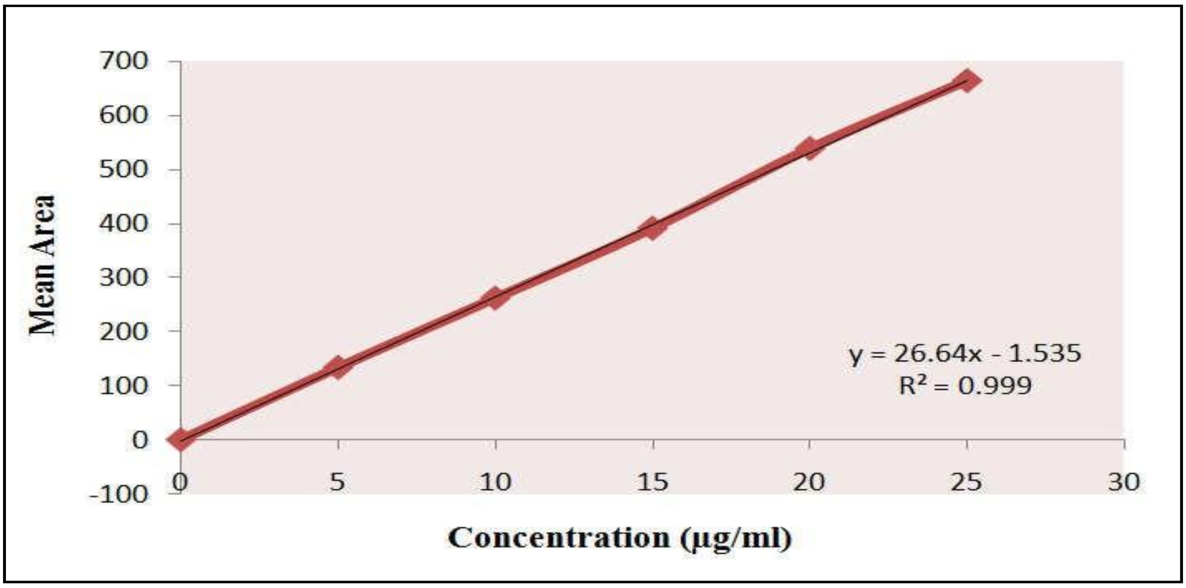
Accurately weighed 10 mg of Dexrabeprazole was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of methanol and sonicate for 10 min., then volume was made up to 50 ml with methanol and vortex it to get complete dissolution and then filtered by whatmann filter paper (no.41). Concentration of Dexrabeprazole in methanol was 200 µg/ml. (stock- A).

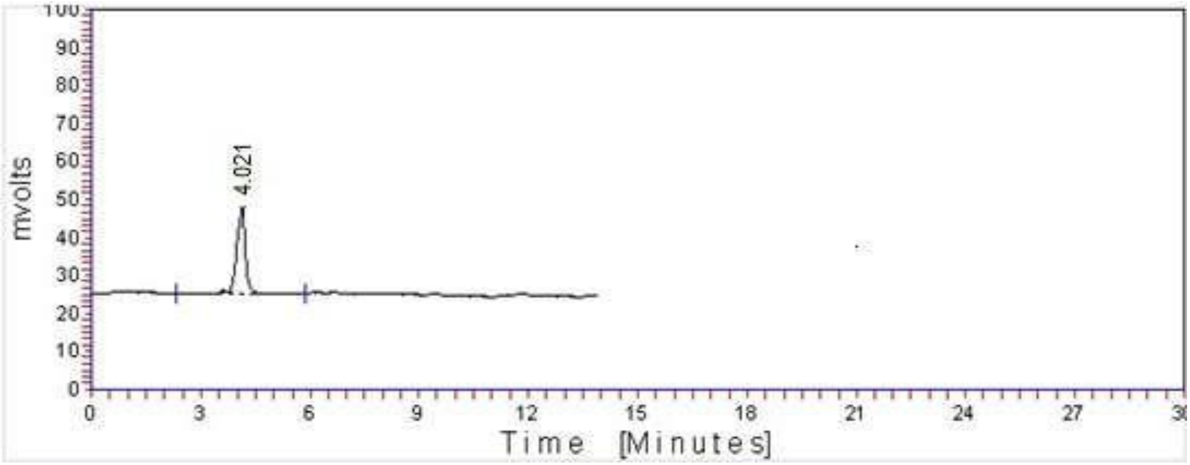
2. Preparation of Sub Stock Solution, 5 ml of solution was taken from stock-A of Dexrabeprazole and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent to give concentration of 100 µg/ml (Stock-B). Experimental Work Page 32.

3. Preparation of Different Solution 0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with methanol. This gives the solutions of, 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of Dexrabeprazole.

Standard Concentratioµg/ml	**Area under Curve (AUC)**						**Mean**
	Rep-1	**Rep-2**	**Rep-3**	**Rep-4**	**Rep-5**	**Rep-6**	
5	135.658	125.658	136.658	137.745	126.658	139.954	133.7218
10	268.895	256.658	249.985	265.587	274.454	255.698	261.8795
15	397.475	385.654	382.145	396.478	390.745	393.321	390.9697
20	532.145	538.854	539.985	539.954	537.662	542.147	538.4578
25	652.325	647.854	662.258	659.985	685.458	674.458	663.7230
Correl Coeff (r^2)							0.999
Slope (m)							26.64
Intercept (c)							-1.535

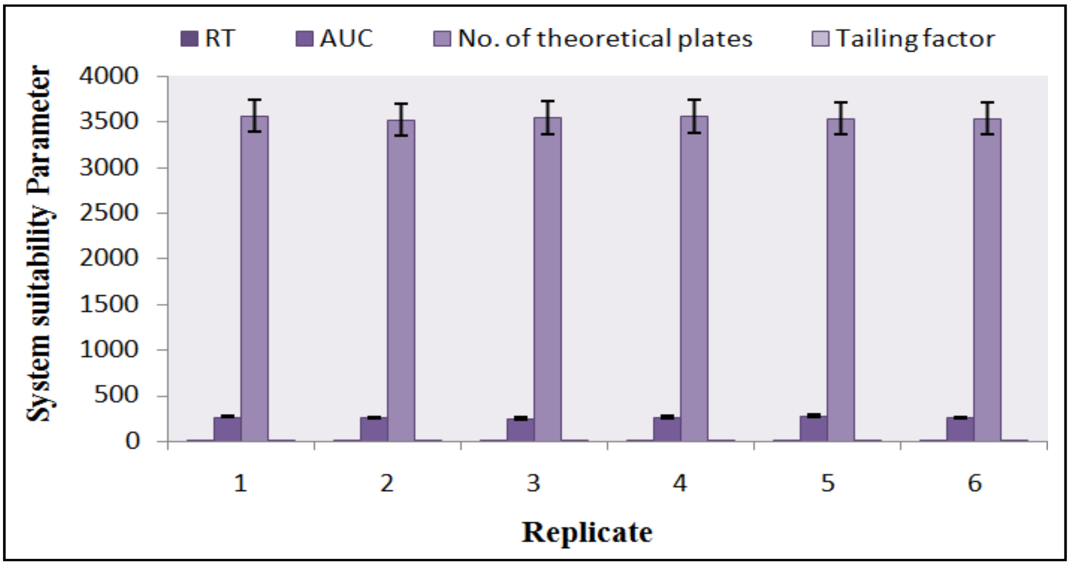
4. Linearity and Calibration Graph to establish: The linearity of analytical method, a series of dilution ranging from 5-25 µg/ml was prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded.





7.3 System Suitability Parameters

System suitability Parameter →	RT	AUC	No. of theoreticalplates	Tailingfactor
Rep-1	4.021	268.895	3565	1.12
Rep-2	4.025	256.658	3525	1.15
Rep-3	4.035	249.985	3545	1.13
Rep-4	4.037	265.587	3562	1.14
Rep-5	4.034	274.454	3541	1.12
Rep-6	4.035	255.698	3540	1.15
Mean	4.031	261.880	3546.333	1.135
S.D.	0.007	9.252	14.962	0.014
% R.S.D.	0.162	3.533	0.422	1.214



Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of 10µg/ml Dexrabeprazole was injected separately. Peak report and column performance report were recorded for all chromatogram. Experimental Work Page 34 Table 7.6: System Suitability Parameters of Dexrabeprazole System suitability Parameter → RT AUC No. of 7.4: System Suitability Parameters of Dexrabeprazole Experimental Work Page 35 7.4.

Validation of Developed Method

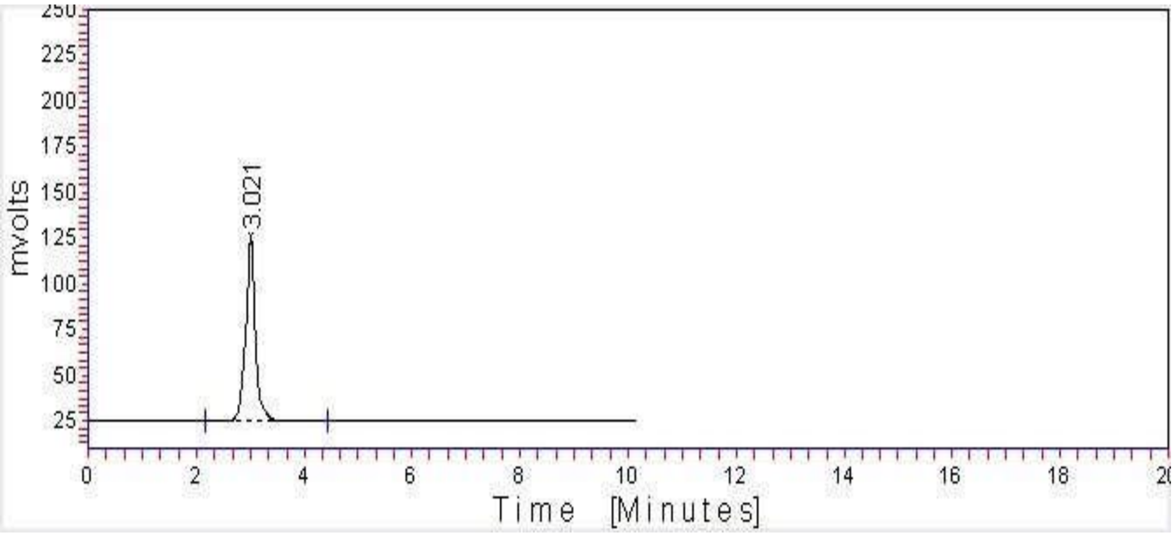
(a) Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure Figure 7.5: Response Ratio Curve of Dexrabeprazole.

Replicates	Concentration (µg/ml)	Mean AUC	Response Ratio
Rep-1	5	133.7218	26.744
Rep-2	10	261.8795	26.188
Rep-3	15	390.9697	26.064
Rep-4	20	538.4578	26.922
Rep-5	25	663.723	26.548
Mean	26.494		
SD	0.363		
%RSD	1.137		

(b) Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components. Figure 7.6: Chromatogram of pure drug Experimental Work Page.



(c) Accuracy

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found. (µg/ml)			% conc. Found			Mean % conc.
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	**4**	4.01	3.99	3.85	100.25	99.50	96.49	98.75
10	**8**	7.85	7.95	7.84	98.13	101.27	98.62	99.34
15	**9.6**	9.74	9.85	9.65	101.46	101.13	97.97	100.19
MEAN								99.42
SD								0.723
% RSD								0.727

(d) Precision

The precision are established in three differences: 1. Repeatability 2. Intermediate precision a) Day to Day b) Analyst to Analyst 3. Reproducibility

I. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25µg/ml for Dexrabeprazole indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively.

CONC.REP.	**CONCENTRATION FOUND (μg/ml)**					**MEAN**
	5	10	15	20	25	
Replicate-1	4.85	9.95	14.85	19.85	24.78	
Replicate-2	4.98	9.85	14.96	19.98	24.65	
Replicate-3	4.78	9.65	14.88	19.96	24.78	
Replicate-4	4.65	9.78	15.02	19.78	24.65	
Replicate-5	4.88	9.95	14.96	19.65	24.74	
MEAN	4.828	9.836	14.934	19.844	24.72	
% MEAN	96.560	98.360	99.560	99.220	98.880	98.516
SD	0.123	0.126	0.068	0.136	0.066	0.104
% RSD	0.127	0.129	0.069	0.137	0.067	0.106

II. Intermidiate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations. Results of day to day intermediate precision for Dexrabeprazole reported in table 7.13.

CONC. REP.	**CONCENTRATION FOUND (μg/ml)**					**MEAN**
	5	10	15	20	25	
Replicate-1	4.95	9.98	14.74	19.96	24.78	
Replicate-2	4.85	9.85	14.85	19.98	24.65	
Replicate-3	4.65	9.78	14.65	19.87	24.74	
Replicate-4	4.99	9.69	14.78	19.85	24.85	
Replicate-5	4.92	9.99	14.96	19.96	24.63	
MEAN	4.872	9.858	14.96	19.924	24.73	
% MEAN	97.440	98.580	99.733	99.620	98.920	98.859
SD	0.134	0.129	0.117	0.059	0.091	0.106
% RSD	0.138	0.131	0.117	0.060	0.092	0.108

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 10Mm KH2PO4: methanol (20:80% v/v), to (20:80% v/v). Results of robustness are reported in table 7.14 Experimental Work Page 41 Table 7.14: Robustness of Dexrabeprazole CONC. REP. CONCENTRATION FOUND (μg/ml).

CONC. REP.	**CONCENTRATION FOUND (μg/ml)**					**MEAN**
	5	10	15	20	25	
Replicate-1	4.85	9.95	14.78	19.96	24.74	
Replicate-2	4.96	9.85	14.65	19.85	24.65	
Replicate-3	4.78	9.78	14.74	19.78	24.78	
Replicate-4	4.95	9.65	14.96	19.62	24.63	

Replicate-5	4.85	9.85	14.85	19.63	24.63	
MEAN	4.878	9.816	14.96	19.768	24.686	
% MEAN	97.560	98.160	99.733	98.840	98.744	98.607
SD	0.076	0.111	0.117	0.145	0.069	0.104
% RSD	0.078	0.113	0.117	0.147	0.070	0.105

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve. Table 7.15: LOD and LOQ OF Dexrabeprazole S. No. LOD (µg/ml) LOQ (µg/ml) 1. 0.65 1.85 7.4.1.

S. No.	LOD (µg/ml)	LOQ (µg/ml)
1.	0.65	1.85

Analysis of drugs in tablets formulation

Tablet powder amount equal to 10mg of Dexrabeprazole was taken in 100ml volumetric flask. This was than dissolve in 25 ml of methanol by sonication for about 10 minutes. The volume is made up to the mark by methanol and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration. Results of tablet analysis are reported in table 7.16. Experimental Work Page 42 Table 7.16: Analysis of Tablet Sample Dexrabeprazole* Label Claim (mg) 10mg % Found (mg) 9.95 % Assay 99.50 % RSD 0.215 *Average of three determination 7.5 Forced degradation studies In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected. 1. Acid degradation: 50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 N HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug. 2. Alkaline hydrolysis: 50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug. 3. Oxidative degradation: 50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug. Experimental Work Page 43.

4. Thermal degradation: 50 mg of the drug sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Stress conditions	**Drug recovered (%)**	**Drug decomposed (%)**
Standard drug	99.95	0
Acidic hydrolysis	82.23	17.77
Alkaline hydrolysis	89.98	10.02
Oxidative degradation	96.65	3.35
Photolytic degradation	91.14	8.86

Table 7.17: Results of Forced degradation studies Stress conditions Drug recovered (%) Drug decomposed (%) Standard drug 99.95 0 Acidic hydrolysis 82.23 17.77 Alkaline hydrolysis 89.98 10.02 Oxidative degradation 96.65 3.35 Photolytic degradation 91.14 8.

RESULT & DISCUSSION

Method Development and Validation for Estimation of Dexrabeprazole in synthetic mixture using RP-HPLC.

The RP-HPLC method was developed for estimation of Dexrabeprazole in combined formulation by isocratically using 10mM KH_2PO_4: methanol in the ratio of 20:80 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 258nm. Then developed method was validated by using various parameters.

System suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10µg/ml of Dexrabeprazole were injected and chromatogram was recorded. The result of system suitability parameter is reported in table 8.1.

Table 8.1: Results of system suitability parameters.

Parameters	**Dexrabeprazole**
No. of Theoretical Plates	3546.333±14.962
Tailing Factor	1.135±0.014
Retention time	4.031±0.007

Linearitys

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 8.2.

Table 8.2: Results of Linearity of Dexrabeprazole.

Parameter	**Dexrabeprazole**
Concentration (µg/ml)	5-25
Correlation Coefficient (r^2)*	0.999
Slope (m)*	26.64
Intercept (c)*	-1.535

*value of five replicate

Specificity

Specificity of the method was determined and the peaks of plasma, diluent, mobile phase and excipient of physical mixture did not interfere with standard peaks of Dexrabeprazole.

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less then 2 indicate the accuracy of method. Result of recovery study shown in table 8.3.

Table 8.3: Results of recovery study.

% Level	**% MEAN±SD***
80%	99.42±0.723
100%	99.87±0.098
120%	99.38±0.977

* Value of three replicate and three concentrations

Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by differentanalyst. The value of SD and %RSD are less then 2 indicate the precision of method. Result of precision shown in table 8.4.

Table 8.4: Results of precision.

Parameter	**% MEAN±SD***
Repeatability	98.516±0.104
Intermediate precision	
Day to day precision	98.859±0.106

***** Value of five replicate and five concentrations

Robustness

The robustness of developed method was checked by changing in the deliberate variation in solvent. Result of robustness shown in table 8.5.

Table 8.5: Results of Robustness.

Parameter	**% MEAN±SD***
Robustness	98.607±0.104

***** Value of five replicate and five concentrations

LOD and LOQ

Detection limit and quantitation limit of described method were observed as 0.65µg/ml and quantitation limit 1.85µg/ml respectively based on the SD of response and slope, which meet the requirement of new method.

Assay of tablet formulation

The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less then 2 indicate the no interference of excipient in the estimation of drug.

Table 8.6: Assay of synthetic mixture.

	Dexrabeprazole *
Label Claim (mg)	**10mg**
% Found (mg)	9.95
% Assay	99.50
% RSD	0.215

*Average of three determination

Results of forced degradation studies

Table 8.7: Results of Forced degradation studies.

Stress conditions	**Drug recovered (%)**	**Drug decomposed (%)**
Standard drug	99.95	0
Acidic hydrolysis	82.23	17.77
Alkaline hydrolysis	89.98	10.02

Oxidative degradation	96.65	3.35
Photolytic degradation	91.14	8.86

9. SUMMARY AND CONCLUSION

In the present research work, a successful attempt was made for "Development of forced degradation method and validation for the estimation of Dexrabeprazole in marketed formulation" which was developed by experimentation based on thoroughliterature survey and ascertained by statistical parameters of sampling.

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in blood plasma.

Liquid chromatographic system from waters comprising of manual injector, waters 515 pump for constant flow and constant pressure delivery and U.V. Vis. Detector connected to data ace software for controlling the instrumentation as well as processing the data generated were used. Drug sample was extracted by precipitating method using 5ml of methanol for each ml of plasma sample. The proposed methods were found to be linear with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent.

The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form.

The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

REFRENCESES

1. Sethi P.D.; HPLC: Quantitative Analysis of Pharmaceutical Formulation; CBS Publishers and Distributors, New Delhi, 1996; 113-202.
2. Davidson A.G., Beckett A.H. and Stenlake J.B.; Practical Pharmaceutical Chemistry; 4th edition; CBS Publishers and Distributors, New Delhi, 1989; 276- 99.
3. Jeffery G.H., Bassett J., Mendham J. and Denrey R.C.; Vogel's Textbook of Quantitative

Chemical Analysis; 5th edition; Longman Group UK Ltd, England, 1989; 6-14.

4. Http, //wwwyoungincom/application/AN-0608-0115ENpdf.
5. Swarbrick, James B. and James. C.; Encyclopedia of pharmaceutical technology; Volume I; Marcel Dekker Inc., New York, 1998; 217 - 224.
6. Sahu R. Nagar P. and Jain D.; Indian J. Pharm. Science, 2006; 68(4); 503-506.
7. Jain N., Jain R., Swami H. and Pandey S.; Indian Journal of Pharmacy and Pharmaceutical sciences, 2009; 1(1): 189-191.
8. Khan, M.R. and Jain, D.; Indian Journal of Pharmaceutical sciences, 2006; 64(6): 546-548.
9. Meyer Veronica R.; Practical High Performance Liquid Chromatography; 2nd edition, John wiley and sons, London, 1993; 56-58.
10. Braumann T., Weber G. and Grimme L.H.; J.Chromatogr, 1983; 48-65.