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Development of assay method and forced degradation study of dexibuprofen and paracetamol by RP-HPLC in tablet formulation

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ABSTRACT

A novel, rapid, specific and stable RP-HPLC assay method was developed and validated for the simultaneous estimation of Dexibuprofen and Paracetamol in tablet dosage form. The separation was carried out by using a mobile phase consisting of Water, Acetonitrile, Methanol and Orthophosphoric acid in the ratio of 300:200:400:1. The column used for separation was Hypersil BDS, C18 (4.6 x 250mm x 5µ) with flow rate of 1.3ml/min and wavelength at 220nm. The retention time for Dexibuprofen and Paracetamol was 10.52 and 2.19 respectively. The stability of the developed method was estimated by stress testing of Dexibuprofen and Paracetamol by exposing them to various forced degradation conditions like acid-base testing, oxidative testing and thermal stress testing. The method was also validated in terms of accuracy, precision, linearity, system suitability, robustness and ruggedness as per ICH guidelines.

Key words: Dexibuprofen, Paracetamol, Assay, ICH, Forced Degradation.

INTRODUCTION

The multidrug therapy is a well-known technique for administration of two more active drug components in a single dosage form, it has better patient acceptability due to reduced number of dosage forms to be taken at a time.Analytical methods are extensively available for single drug formulations but due to complexity in multicomponent formulations, method development for multiple drugs formulation is challenge and scope for new developments. The present research work is aimed to develop a proper solvent system and method development by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) for the analysis of multi-drug combination including Dexibuprofen and Paracetamol and to validate the developed process as per the ICH guidelines.Dexibuprofen,(S+ Enantiomer of Ibuprofen) is (S-2-(4-lsobutylphenyl)-propionic acid[1] comes under the classification of Non-steroidal Anti-inflammatory Drug (NSAID).It is commonly used in the symptomatic treatment for osteoarthritis, muscular-skeletal pain, primary dysmenorrhea, dental pain etc. It's analgesic and anti-inflammatory effects are better than its racemic Ibuprofen, it also reduces gastric damage [2].Various analytical techniques have been used for the estimation of Dexibuprofen, High performance thin layer chromatography (HPTLC)[3], RP-HPLC method [4], Densitometric analysis of 2-aryl propionate derivatives in pharmaceutical preparations [5].The molecular formula of Dexibuprofen is $C_{13}H_{18}O_2$ and molecular weight is 206.28, its chemical structure is shown in figure 1.

Paracetamol, [N-(4-hydroxyphenyl) acetamide], is a NSAID[6, 7]. It is widely used for its antipyretic and analgesic effect, but has lesser anti-inflammatory action which acts by inhibiting the synthesis of prostaglandins[8, 9, 10]The molecular formula of Paracetamol is $C_8H_9NO_2$and molecular weight is 441.90,the structure of Paracetamol is shown in figure 2.

There are various spectrophotometric methods reported in which Paracetamol alone or in combination with other drugs had been estimated. [11, 12, 13, 14]

As this method is not available in any Pharmacopeia, so we had attempted to develop a simple, accurate, stable and economical analytical methodfor the estimation of Dexibuprofen and Paracetamol in combined dosage form and validate it according to International Conference on Harmonization (ICH) guidelines.This paper describes validated RP-HPLC method for simultaneous estimationof Dexibuprofen andParacetamol incombination usingthe mobile phase as Water: Acetonitrile: Methanol: Orthophosphoricacid in the ratio of (300:200:400:1).

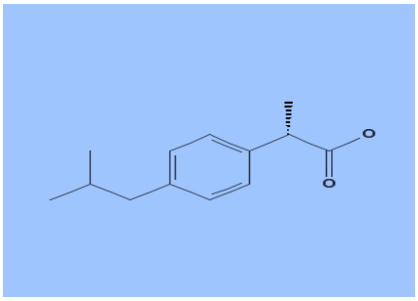

Figure 1: Structure of Dexibuprofen

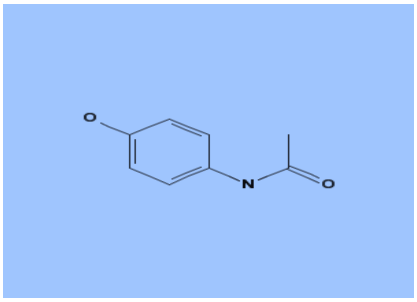

Figure 2: Structure of Paracetamol

MATERIALS AND METHODS

Reagents and Chemicals: All chemicals used were of HPLC grade. Dexibuprofen pure was obtained as a gift sample from Noven Life sciences (P) Ltd, Hyderabad, Paracetamol was obtained as a gift sample from Suven pharmaceutical, Hyderabad.

Instrumentation and Chromatographic Conditions: High performance liquid chromatography (RP-HPLC) was performed on Waters 2487 HPLC system that comprises with Empower 2 software,the column used for separation was Hypersil BDS, C18 (4.6 x 250mm x 5µ). The elution was carried out isocratically[15].

Table 1: Optimized ChromatographicConditions

	Parameter	Result
1	Column	Hypersil BDS , C18 (4.6 × 250mm x 5µ)
2	Mobile phase	Water:Acetonitrile:Methanol:Orthophosphoric Acid (300:200:400:1)
3	Flow rate	1.3 ml/min
4	Detection Wavelength	220 nm
5	Injection Volume	20 µl
6	Run Time	15 min
7	Temperature	30^0 C

Preparation of Standard Stock Solution: Weighed accurately 150 mg of Dexibuprofen(working standard) and 250mg of Paracetamol (working standard) and were dissolved in 70ml of Methanol and sonicated for 45 minutes at cooling conditions, then the volume was made up to 100ml with methanol and further dilute 5 ml of this solution to 100 ml with the mobile phase.

Preparation of sample solution: The sample solution was prepared by taking 20 tablets of Dexibuprofen and Paracetamol respectively and wascrushed. Thecrushed powder was weighed, equivalent to about 150 mg of Dexibuprofen and 250 mg of Paracetamol, then dissolved in 70ml of methanol and sonicated for 45 minutes at cooling conditions and diluted to 100ml with methanol.Further, diluted 5ml of solution to 100ml with the mobile phase.

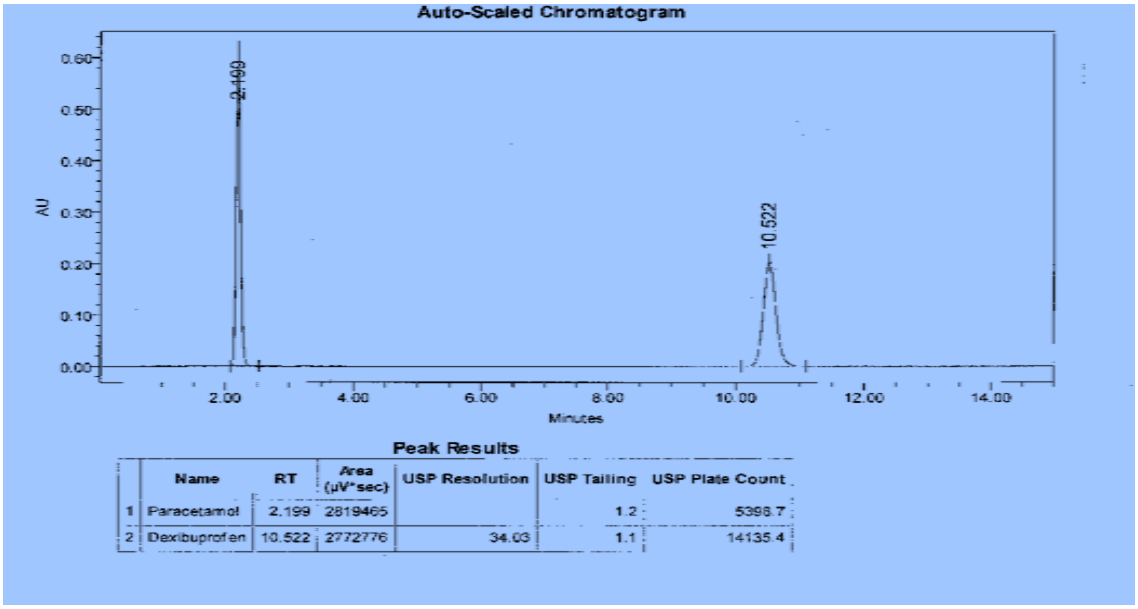

Figure 3: HPLC Chromatogram for the mixture of Dexibuprofen and Paracetamol in pharmaceutical formulation

ANALYTICAL METHOD VALIDATION

Validation of the developed RP-HPLC method for simultaneous estimation of Dexibuprofen and Paracetamol was performedby following parameterstudies [16].

Linearity and Range

Linearity is the ability of the analytical method toobtain results that are directly proportional to the concentration of the analyte in the sample. The range of an analytical method is the interval between the upper and lower levels of analyte that have been determined with precision, accuracy and linearity. Linearity was determined by preparing five standard solutionsof Dexibuprofen and Paracetamol standard at concentration levels of 60% to 140% of test concentration and each solution was injected to be confirmed. Peak areas were recorded and calibration plots were constructed, the drug response was found to be linear, observation shown in table 2, the calibration curves were shown ion figure 4 and 5 for Dexibuprofen and Paracetamol, respectively.

Table 2: Linear Regression data for Calibration curve of Dexibuprofen and Paracetamol

S.No	Drug	Linearity Range (µg/ml)	Slope	Intercept	Correlation coefficient
1	Dexibuprofen	60-140	37.1	3.2	0.9999
2	Paracetamol	60-140	23.7	16.4	0.9999

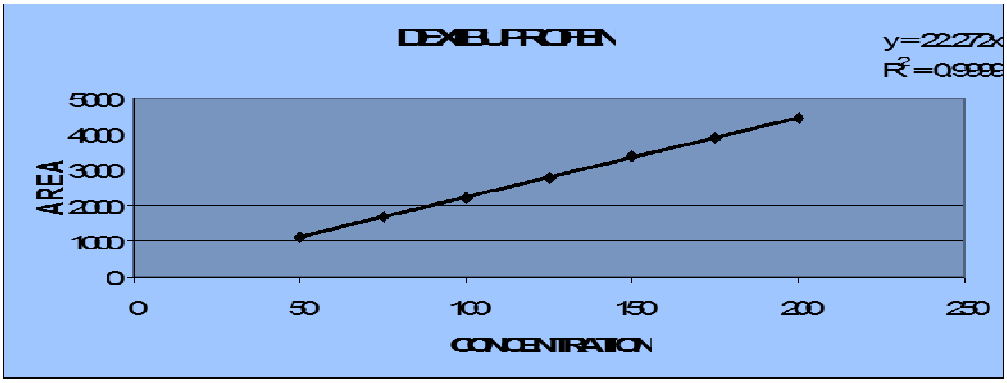

Figure 4: Calibration curve of Dexibuprofen

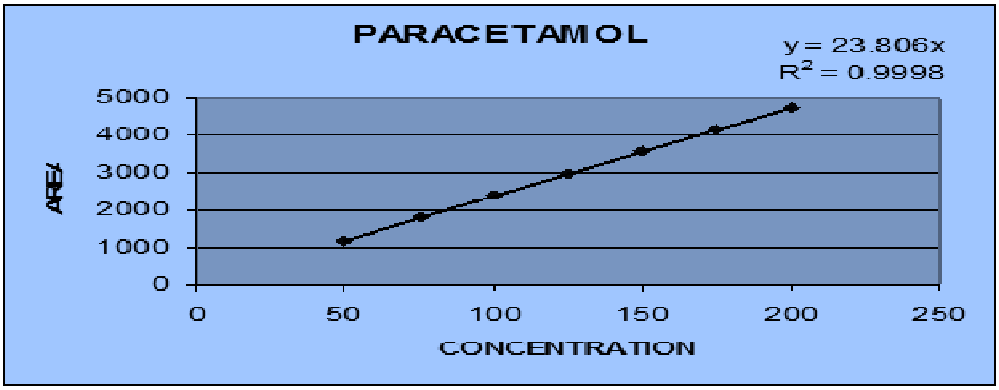

Figure 5: Calibration curve of Paracetamol

Precision

The 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 under the prescribed condition. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation. The system precision i.e. also called injection repeatability was performed by preparing standard stock solution as per test method and injected six times. The observations were shown in table 3.

Table 3: Observation of System Precision

Concentration	Injection	Area	Statistical Analysis
100 %	Injection 1	2767.83	Mean : 2772.93
	Injection 2	2773.60	
	Injection 3	2773.57	SD : 3
	Injection 4	2773.54	
	Injection 5	2774.68	RSD : 0.11%
	Injection 6	2774.38	

(Note: Acceptance Criteria: RSD should not be more than 2%)

Table 4: Observation of Sample Precision forDexibuprofen

Concentration of Dexibuprofen	Area	% Assay	Statistical Analysis
100 %	4180	100.23	Mean: 100.40
	4207	101.46	
	4184	100.88	SD: 0.285
	4185	100.88	
	4175	100.67	RSD: 0.28%
	4195	101.18	

(Note: Acceptance Criteria: RSD should not be more than 2%)

Table 5: Observation of Sample Precision for Paracetamol

Concentration of Paracetamol	Area	% Assay	Statistical Analysis
100 %	4161	98.55	Mean: 98.84
	4198	99.44	
	4162	98.58	SD: 0.344
	4184	99.06	
	4167	98.70	RSD: 0.35%
	4168	98.73	

(Note: Acceptance Criteria: RSD should not be more than 2%)

The test procedure can be considered to be precise in terms of system precision as the data was within for the assay of Dexibuprofen and Paracetamol.

The method precision i.e. sample repeatability wasperformed by taking six different samples and prepared as per test method and injected. The obtained data were shown in the table 4 and 5.

From the above data it can be concluded that the test procedure can be considered to be precise in terms of sample repeatability for the assay of Dexibuprofen and Paracetamol.

Accuracy

Accuracy is a measure of exactness of the analytical method. It was performed by preparing solutions at varying levels 80 %, 100% and 120 % of test concentration using Dexibuprofen and Paracetamol Standard solution and added to placebo. Each solution was injected three times.The accuracy of the method was determined by spiking known amount of Dexibuprofen to placebo at 80%, 100% and 120% of test concentration in triplicate and analyzing as per the proposed method. The results were shown in Table 6 and 7 for Dexibuprofen and Paracetamol respectively. As the analytical method meets the pre-established acceptance criteria for Recovery study as per protocol, hence the method is accurate.

Table 6: Recovery Study Table for Dexibuprofen

Recovery Level	Amount of Dexibuprofen Added in mg	Actual mg. Of Dexibuprofen added	Amount of Dexibuprofen Found in mg	% Recovery
80	240.29	239.73	240.2	100.18
80	240.33	240.29	240.5	100.08
80	240.34	240.34	241.1	100.32
100	300.26	300.26	299.8	99.84
100	300.19	300.19	300.1	99.96
100	300.27	300.27	300.7	100.15
120	360.12	360.12	358.3	99.49
120	360.28	360.28	360.9	100.18
120	360.29	360.29	358.1	99.38
Mean				99.95
SD				0.30
RSD				0.30

Table 7: Recovery Study Table for Paracetamol

Recovery Level	Amount of Paracetamol Added(mg)	Actual amount of Paracetamol added (mg)	Amount of Paracetamol Found (mg)	% Recovery
80	400.39	399.79	397.1	99.31
80	400.49	399.89	398.2	99.57
80	400.58	399.98	405.6	101.41
100	500.37	499.61	493.3	98.74
100	500.5	499.75	493.8	98.82
100	500.53	499.78	495.4	99.12
120	600.17	599.27	588.3	98.16
120	600.3	599.40	593.2	98.96
120	600.3	599.40	588.0	98.10
Mean				99.13
SD				0.93
RSD				0.93

Ruggedness

It is the degree of reproducibility of test results obtained by the analysis of the same samples under variety of normal test conditions.The ruggedness was determined by analysis of aliquots from homogenous lots in different laboratories, by different analysts, using operational and environmental and conditions that may differ but are still within the specified parameters of the assay. The degree of reproducibility was compared to the precision of the assay under normal conditions to obtain a measure of the ruggedness of the analytical method. Samplesolutions were prepared as per test method by two different analysts and each solution was injected six times. The result of analysis was shown in table 8.

Table 8: Ruggedness studies

S.No.	Drug	Label Claim (mg)	Amount Found %	
			Analyst 1	Analyst 2
1	Dexibuprofen	150	100.91	100.72
2	Paracetamol	250	99.62	99.37

Robustness

The robustness of the method was evaluated by deliberately varying the chromatographic conditions i.e. composition of organic phase in mobile phase by ± 2% absolute, flow rate by ± 0.2ml, column oven temperature by ± 5^o C, change in pH of mobile phase by ± 0.2 units and change in wavelength of detection by ± 2nm. At these altered conditions the standard and sample solutions were injected. The system suitability was evaluated in each varied condition. The amount of Dexibuprofen and Paracetamol was calculated from sample solution in each varied condition. Results were tabulated in table 9 and 10 indicatesthat the method is robust under varied conditions. For the acceptance criteria the overall RSD should be not more than 2.0%.

Table 9: Robustness study data for Dexibuprofen (*)

Method Precision data		**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Set 6**	**Set 7**	**Set 8**
100.23	100.88	99.51	99.53	100.09	100.27	101.44	101.30	100.22	100.48
100.30	100.31	98.81	99.87	99.95	100.30	98.70	98.74	99.98	99.93
100.10	100.60	99.19	100.56	100.78	101.06	99.49	99.18	100.64	101.04
Mean	**100.40**								
SD	**0.285**								
RSD%	**0.28**								
Overall Mean		**99.88**	**100.26**	**100.74**	**100.83**	**100.61**	**100.45**	**100.75**	**100.81**
Overall SD		**0.830**	**0.404**	**0.480**	**0.387**	**0.920**	**1.213**	**0.450**	**0.437**
Overall RSD %		**0.83**	**0.40**	**0.48**	**0.38**	**0.91**	**0.21**	**0.45**	**0.43**

Table 10: Robustness study data for Paracetamol (*)

Method Precision data		**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Set 6**	**Set 7**	**Set 8**
98.55	99.44	98.83	100.23	99.73	101.88	101.62	101.03	99.18	98.00
98.58	99.06	100.15	101.42	99.83	99.77	99.03	98.12	99.22	98.74
98.70	98.73	98.58	100.42	101.23	100.94	98.95	98.35	99.83	98.80
Mean	**98.840**								
SD	**0.344**								
RSD%	**0.350**								
Overall Mean		**98.96**	**99.46**	**99.47**	**99.67**	**99.34**	**99.10**	**99.18**	**98.89**
Overall SD		**0.530**	**1.014**	**0.778**	**1.075**	**0.900**	**0.855**	**0.369**	**0.450**
Overall RSD %		**0.54**	**1.02**	**0.78**	**1.08**	**0.91**	**0.86**	**0.37**	**0.46**

*Where**(*)***

Set 1: *Change in Organic composition in Mobile Phase by + 2% absolute*
Set 2: *Change in Organic composition in Mobile Phase by – 2% absolute*
Set 3: *Change in flow rate by + 0.2 ml/ min.*
Set 4: *Change in flow rate by-0.2 ml/min*
Set 5*: Change in column temperature by + 2.0 °C*
Set 6*: Change in column temperature by - 2.0 °C*
Set 7*: Change in wavelength by +2.0nm.*
Set 8*: Change in wavelength by -2.0nm*

Table 11: System Suitability Studies

S.No.	Parameter	Result
1	Retention time for Paracetamol	2.199(About 2 minutes)
2	Retention time for Dexibuprofen	10.522(About 10 minutes)
3	USP tailing for Paracetamol	1.2 (Not more than 2)
4	USP tailing for Dexibuprofen	1.1 (Not more than 2)
5	Theoretical plates for Paracetamol	5435 (Not less than 2000)
6	Theoretical plates for Dexibuprofen	14194(Not less than 2000)
7	Resolution between the peak of Paracetamol and Dexibuprofen	34.03(Not less than 5)

System Suitability Parameters

Systemsuitability study[17] was performed on freshly prepared standard stock solution of Dexibuprofen and

Paracetamol of both drugs, under optimized chromatographic conditions and following parameters were studied to evaluate the suitability of the system. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and the results are expressed in Table 11.

Stress Study

As per ICH a degradation product is defined as a chemical change in drug molecule brought about over time and by action of light, temperature, pH, or water, or by reaction with an excipient and/or the immediate container/closure system. Degradation samples are analyzed at the initial phases of HPLC method development using purity and potency methods.

Acid/ Base Stress Testing

Acid/Base stress testing is performed to force the degradation of a drug substance to its primary degradation products by exposure to acidic and basic conditions over time.Acid testing was done by taking tablet samples and was treated separately with 5.0ml of 1.0N Hydrochloric acid and kept on bench top for 60 minutes. The treated sample was analyzed and tabulated. For base degradation study, tablet samples was treated separately with 5ml of 1.0N Sodium hydroxide and kept on bench top for 60 minutes. The treated samples wereanalyzed as per the proposed method and results were shown in table 12 and 13.

Thermal Stress Testing

The thermal stress study was carried out with the sample solution at 80°C for 12 hours. Then the sample was screened for degradation products by the developed HPLC method.Data represented in table 12 and 13.

Oxidative Stress Testing

Oxidative studies were performed by taking H_2O_2, tablets sample was treated separately with 5ml of 3.0%v/v solution of hydrogen peroxide and kept on bench top for 60 minutes. The treated sample was analyzed as per the proposed method and results were shown in Table 12 and 13.

Photostability

UV and Visible light are the most energetic electromagnetic radiation sources to which pharmaceutical drug substances and drug products are generally exposed. The sample and control were exposed to both the cool white fluorescent and near ultraviolet lamp. ICH guidelines specify an exposure of 5 X and 10 X 200 watt h/m^2 for solid drug substances. ICH guidelines specify an exposure of 1.2 X 10^6 lux hours for fluorescence.

Table 12: Forced Degradation for Dexibuprofen

Mode of Degradation	Condition	Period	% Assay	% Degradation as compared with Control
Control sample	No treatment	-	98.49	-
Acid	1.0N HCl	10 Minutes	100.39	-1.90
Base	0.05N NaOH	5 Minutes	97.59	0.90
Oxidative	3.0%v/v H_2O_2	1 Hour	81.00	17.49
Thermal	80^0C	12 Hour	101.34	-2.85

Table 13: Forced Degradation for Paracetamol

Mode of Degradation	Condition	Period	% Assay	% Degradation as compared with Control
Control sample	No treatment	-	99.03	-
Acid	1.0N HCl	10 Minutes	100.25	-1.22
Base	0.05N NaOH	5 Minutes	98.07	0.96
Oxidative	3.0% v/v H_2O_2	1 Hour	85.36	13.67
Thermal	80^0C	12 Hour	100.45	-1.42

RESULTS AND DISCUSSION

From the above performed experiment an appropriate assay method was developed for the simultaneous estimation of Dexibuprofen and Paracetamol and subsequently validated as per ICH guidelines. Various preliminary tests were conducted in order to select suitable and optimum conditions, parameters like wavelength detection, development of ideal mobile phase and concentration of the standard solution were determined. The proposed method was simple, rapid and statistically validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the tablet excipients did not interfere in analysis of drugs. The calibration curve showed linearity over a concentration range of 60% to 140%for both drugs as shown in figure 4 and 5 and was linear with a correlation coefficient of

0.9999 and 0.9998for Dexibuprofen and Paracetamol respectively, data represented in table 3. For the developed assay method the system precision and the method precision was precise as seen from table 4 and 5 respectively. Since the analytical method meets the pre-established acceptance criteria for Recovery study as per protocol, hence the method is accurate, data showed in table 6 and 7. In method validation, intra and inter day variation were well within limit and was found to be precise with % RSD less than 2, results are tabulated in table 9 and 10 indicates that the method is robust under varied conditions. The System Suitability data was also within the acceptable limit as shown in table 11. The most essential stress studies or the forced degradation studies were performed at various specified conditions as per ICH guidelines and were found to be with the acceptable range. The forced degradation data were shown in table 12 and 13 for Dexibuprofen and Paracetamol respectively.

CONCLUSION

The developed assay method and its validation parameters including forced degradation studies revealed that the method was novel, simple, economical, rapid and stable, hence, it can be concluded that the proposed method was very accurate and can be utilized for routine analysis of these two drugs i.e. Dexibuprofen and Paracetamolin combination instead of processing each drug separately.

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