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ISOCRATIC RP-LC METHOD FOR COMBINED QUANTIFICATION OF THE ETODOLAC AND DEXAMETHASONE IN COMBINATION TABLETS

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***Abstract*:** It has been developed and validated an accurate, sensitive, precise, quick, and isocratic reverse phase HPLC (RP-HPLC) technique for combined quantification of Etodolac, and Dexamethasone in raw materials and pharmaceutical combined dispensing kit contains tablet dosage forms. With acetonitrile as the organic solvent, the best separation was achieved on a 250 mmx4.6 mm i.d., 5µ-particle size Inertsil®-Octadecyl-silyl-3V-Reverse-Phase-C_{18}-column with acetonitrile as the non-polar-modifier and *ortho*-phosphoric acid (0.1%v/v) in water in the isocratic mode of elution as mobile phase solvent at a speed of 1.0 mL.min^{-1}. UV detection was at 245-nm. Etodolac's was 2.0 minutes, and Dexamethasone's was 2.9 minutes. With a correlation coefficient of about 0.9999, peak-response was obtained as function of concentration over the range of 1.6-9.6 µg/mL for dexamethasone and 60-360 µg/mL for etodolac. Etodolac, and Dexamethasone were shown to have a percentage assay of 99.57, and 99.97, respectively. Etodolac, and Dexamethasone each have a limit of detection of 0.2 mcg/mL and 0.0016 mcg/mL, respectively. Etodolac, and Dexamethasone each have a limit of quantification (LOQ) of 0.6 mcg/mL and 0.0048 mcg/mL, respectively. The presence of excipients in the formulation had no effect on the assay method. The procedure is appropriate for use in QC- laboratories since it is quick and precise.
***Key words*:** Etodolac, Dexamethasone, Isocratic-RP-HPLC, and Fixed dosage forms.

INTRODUCTION: Etodolac is a non-steroidal anti-inflammatory derivative (NSAID) that has antipyretic and analgesic properties due to its status as a pyrano-carboxylic acid. An example of a monocarboxylic acid, etodolac is acetic acid with a 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]-indol-1-yl group bonded to one of the methyl hydrogens. It is used for the treatment of rheumatoid arthritis, osteoarthritis, and the relief of post-surgical pain. If given as the racemate, only the (S)-enantiomer will have any effect. Dexamethasone is a synthesized adrenal corticosteroid that effectively reduces inflammation. Dexamethasone is a fluorinated steroid that contains OH-groups at positions 11th /17th -&-21st, a CH_3-group at position 16, and (=O) groups at positions 3-&-20. It is structurally a 9-fluoropregna-1,4-diene. This compound is known by many other names, including fluorinated steroid, glucocorticoid, 3-oxo-Δ-(1) steroid, Δ-(4) steroid, 20-oxo steroid, 11-β-OH-Steroid, 17-α-OH-steroid, and 21-OH-Steroid. Dexeto® pills are in-house fixed tablet dosage form containing 300-mg of Etodolac and 8mg of Dexamethasone. Nevertheless, no techniques for combined determination of -Etodolac & Dexamethasone in oral fixed dosage form have been published. Furthermore, no official or preliminary monograph on this combination of analytes has been published in any of the compendial pharmacopoeias.[9] The goal of this study was to develop a accurate and efficient RP_HPLC method to estimate a new combination of anti-inflammatory drugs in fixed dosage forms for oral administration. The validation of the devised approach is also addressed in this study, as per ICH standards [10]

Experimental: <u>Chemicals and Reagents:</u>

- 99%, Etodolac of 99% and Dexamethasone of 99% pure are acquired from Sigma-Aldrich Chemicals, Mumbai, India.
- Rankem-Fine-Chemicals of HPLC- Grade- Acetonitrile
- *ortho*-H_3PO_3, 85% (v/v) obtained from Quligen-Fine chemicals.
- Chromatographic-Grade water

<u>Chromatographic-Instrument:</u> Quantitative HPLC was carried out on a Waters Corporation liquid chromatograph, model number 2695, fitted with either a 2489 UV/visible detector or a 2998 PDA detector, which included an automated injector with a 20 microliters injection volume and a quadra-pump. The column utilized was a Reverse_Phase_Inertsil-Octa-Decyl-S-3V-C_{18} column (250mmx4.6 mm internal diameter with particle size 5µm). Spin-chrome Software was installed on the HPLC equipment. The column temperature-40° was adjusted and eluted over 20.0 minutes at a mobile solvent speed of 1.0 mL.min^{-1} under isocratic conditions. The

organic modifier is acetonitrile, while the mobile phase is water with *ortho*-phosphoric acid (0.1 percent v/v). (0.1% v/v). It was degassed and filtered via 0.45-m Nylon membrane filters before use. For both the analytes, UV detection at 245 nm was used as isobestic wavelength detection with a PDA detector. CH_3CN:H_20 in a ratio of 50:50 (v/v) is used as diluent to make the standard dilutions. Etodolac was eluted at 2.09 mins and dexamethasone at 2.99 minutes

Preparation of the Primary Standard Drug solutions: To make the primary standard stock solution, 300mg of Etodolac, and 8mg of Dexamethasone were dissolved in a volumetric flask (100mL) with 20mL of diluent (50:50 v/v CH_3CN: H_2O), sonicated for 15 minutes, and then brought up to 100mL with diluents to get the primary standard stock solution containing 3000µg-$mL^{-1}$of Etodolac and 80µg-$mL^{-1}$of Dexamethasone.

Preparation of Working Standard Drug Solution: After adding 5 ml of the primary working standard solution to the 50-mL volumetric flask, the flask was filled with 50 ml of water. This resultant mixture, which includes 300 ug/mL of etodolac and 8 ug/mL of dexamethasone, is suitable for use as a working standard solution. The stock solutions were kept in a cool, dark place that was controlled to be four degrees Celsius.

Sample Preparation: After measuring the weight of each individual tablet, we were able to calculate the average weight of twenty Dexeto® pills. Crushing the tablets into a powder form obtained a sample containing 300-mg of Etodolac and 8mg of Dexamethasone, which was then weighed, shifted to a 100mL pre-calibrated-measuring flask, and dissolved in a blend of acetonitrile and aqueous media with a volumetric ratio of 50:50 (v/v). After being sonicated in diluent and strained via Whattman#41 filter paper, the resultant primary working sample solution has 80mcgs-mL^{-1} of Etodolac and 3000 mcgs-mL^{-1} of Dexamethasone. After quantitatively transferring 5mL-filtrate to a 50-mL pre-calibrated-measuring flask, the diluents were added to bring the volume of the solution to 50 mL. This mixture serves as a working testing solution has 300 mcgs-$mL^{-1}$of Etodolac and 100mcgs-$mL^{-1}$of Dexamethasone. The stock solutions were kept in a dark place at 4 degrees centigrade.

Discussion and Results The purpose of this research was to create a chromatographic technique for the separating and quantifiable determination of fixed-dose Etodolac and Dexamethasone

Optimized Chromatographic Conditions:

Elution solvents	: Elution solvents are acetonitrile. 0.1% *Ortho*-H_3P0_3 acid in H_20: Acetonitrile (40:60 vol-by-vol)
Elution mode	: Isocratic-
Column	: Inertsil ODS C-18-3V (250x4.6mm, 5µm particle size)
Flow rate	: 1.0 ml/min
Injection volume	: 20 µl
Detector	: Photo diode array (PDA)
Wavelength (λ_{max})	: 245nm
Column temperature	: Ambient
Diluent	: CH3CN and H20 in the ratio of 50:50 (v/v)
Run time	: 7 minutes
Retention time	: Etodolac-2.09min and Dexamethansone-2.92

Linearity: Aliquots of Etodolac and Dexamethasone working stock solutions were placed in various 10mL volumetric flasks and made the volume up to the 10mL with the mobile phase, yielding in final strengths of 4-24µg.mL^{-1} and 20-120 µg.mL^{-1}, respectively (Table 4). The peak areas and retention times of each of these drug solutions (loaded at 20µL) were measured thrice in the column. Using a PDA-detector set at 245 nm, a linearity-graph was generated by plotting peak areas-vs- Etodolac & Dexamethasone concentrations in µg-mL^{-1}

Accuracy: The approach's accuracy was found by evaluating the drugs' recovery using the standard-spiking method. To assess if the analytes contained in the formulation caused positive or negative interventions, known amounts of each drug equivalent to 10 percent standard drug solution were added to 80 percent, 100 percent, and 120 percent of the target test concentrations a formulation mixture. Each set-of-addition was replicated thrice at each dilution levl. The results are compared to a competent reference standard after extraction of sample preparation. The percentage of analytes re-covered by the assay was used to assess the accuracy. Table- shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision: Quality-control samples in 100 % (w/v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability is indicated by the low RSD value (1%). (Table-3)

Limits of Detection and Quantification: The method's LOD was set at the lowest concentrations of active pharmaceutical components with a signal-to-noise (S/N) ratio of around 3. (LOD). The lowest active therapeutic medication concentrations that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ).

Method Applicability: Our study group evaluated the newly created method by applying it to pharmaceutical tablets for the estimate of Etodolac, and Dexamethasone

RESULTS AND DISCUSSION: ***Optimization of Chromatographic Conditions:*** A isocratic RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, reproducible, and quick-to-use method for the determination of Etodolac, and Dexamethasone concentrations in formulary matrices. We examined the effect of various HPLC technique variables on the result of the study to optimize the chromatographic parameters, various proportions of CH_3CN: *O*-H_3PO_3, CH_3CN-H_2O, and CH_3CN-KH_2PO_4 buffer were tested. After several early investigatory tests, CH_3CN: *O*-H_3PO_3 (0.1%) binary system at the proportion of 50:50 (vol-by-vol). It was chosen over other mobile phases because it resulted in improved resolution of active components. This procedure gives the good separation of analytes after multiple exploratory & investigatory trail runs. All three active pharmaceutical analytes had excellent UV sensitivity and were interference-free at 245 nm. The analyte peaks were highly defined and without any incidence of tailing under these

conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method Validation Tests: Method precision (RSD, percent), method accuracy (recovery percent & %RSD,), linear range (r^2), and LOD & LOQ were explored as recommended method validation characteristics.

Linearity: With a correlation coefficient of 0.999, the graph of chromatographic-peak areas of all analytes versus respective concentrations was shown to be linear in the band of 60-360 µg. mL^{-1} for Etodolac, and 1.6-9.6 µg.mL^{-1} for Dexamethasone (Table 4). The least square fit data of linear regression analysis was derived from the measurements is given in Table I. Etodolac's is **y = 6227x**, and Dexamethasone's is **y= 858550x**. Table 1 presents the regression parameters for this technique that include slope, intercept, and % RSD. These findings suggest that there was a significant correlation.

Accuracy: Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 89.1 percent to 98.98 percent, for etodolac and 89% to 98.77% for dexamathasone demonstrating the method's accuracy. The RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 2)

Precision: Table 3 summarizes the intraday and interday fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (lessthan-1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs (Table 3).

Robustness: At three different levels, –ve-2, 0, and +ve2, the influence of minute but intentional alterations in the separation parameters was explored. The experimental settings were purposely changed at two distinct levels to determine the robustness of this approach, and the RT and chromatographic responses were evaluated. The effect was observed by modifying one factor at a time. The RT and response of the method were not affected by changing the stationary phase, or mobile phase flow rate by 1.0 $mL.min^{-1}$ (0.8 & 1.2 $mL.min^{-1}$, demonstrating that the procedure was robust. Table 5 summarises the findings.

Limit-of-Detection & Limit-of-Quantifications: Etodolac, and Dexamethasone each have a limit of detection of 0.2 mcg/mL and 0.0016 mcg/mL, respectively. Etodolac, and Dexamethasone each have a limit of quantification (LOQ) of 0.6 mcg/mL and 0.0048 mcg/mL, respectively. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify analytes over a wide concentration range.

Specificity: The RTs for Etodolac, and Dexamethasone were determined to be 2.09 minutes for Etodolac, and 2.89 minutes for Dexamethasone, according to the representative chromatogram given in Figure 1. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signals was observed in the respective RTs of the chromatogram. It indicates that the analytes were not disturbed of probable merging peaks. As a result, this technique can be employed with certainty.

Table 1: Regression analysis & Operating-System Suitability Results:

Study-Parameter	Etodolac	Dexamethasone
Retention Time (min)	2.08	2.98
Peak areas	1823029	6844111
Percentage of peak areas	21	79
USP-Tailing	1.44	1.35
Theoretical Plates	8233	8395
Resolution	--	0.65
Linear range in (µg/mL)	60-360	1.6-9.6
Limit-of-Detection_ in_ µg.mL^{-1}	0.2	0.0016
Limit-of-Quantification in_µg.mL^{-1}	0.6	0.0048
Correlation-Coefficient (r^2)	0.999	0.999
Assay-in-Percentage (%)	97.25	98.85

Table 2: Summery of the standard calibration Curve for Linearity experiment

Calibration Standard Dilution Level	Concentration of Etodolac (µg/mL)	Peak Area	Concentration of Dexamethasone (µg/mL)	Peak Area
20 %	60	400908	1.6	1488301
40 %	120	786337	3.2	2826767
60 %	180	1150168	4.8	4232199
80%	240	1500421	6.4	5628929
100 %	300	1847097	8	6819799
120 %	360	2222982	9.6	8091968

Table 3: Accuracy evaluation by Spike-analysis method

Accuracy study at 80% target level	Injection Number	Etodolac		Dexamethasone	
		Standard Soln.	Spiked Soln.	Standard Soln.	Spiked Soln.
Dexeto-® tablet dosage form solution at 80% level was spiked with 10% of mixed standard solution of API's	1	1527836	1687308	5579053	6179914
	2	1514904	1684077	5549770	6180579
	3	1511818	1668650	5559725	6195603
	Mean area	**1518186.0**	**1680011.7**	**5562849.3**	**6185365.3**
	Std. Dev	8498.4	9971.2	14889.4	8872.3
	% RSD	**0.6**	**0.6**	**0.3**	**0.1**
	%Recovery	**88**		**89**	
80% of the target concentration is equivalent to Etodolac-240µg/mL and Dexamethasone- 6.4µg/mL in acetonitrile: water 50:50 v/v as diluent.					
Accuracy study at 100% target level	Injection Number	Etodolac		Dexamethasone	
		Standard Soln.	Spiked Soln.	Standard Soln.	Spiked Soln.
Dexeto-® tablet dosage form solution at 100% level was spiked with 10% of mixed standard solution of API's	1	1872831	2017749	6787234	7418260
	2	1854355	2024096	6773832	7450923
	3	1843434	2022646	6758482	7465440
	Mean area	**1856873.3**	**2021497.0**	**6773182.7**	**7444874.3**
	Std. Dev	14859.4	3325.8	14387.0	24164.6
	% RSD	**0.8**	**0.2**	**0.2**	**0.3**
	%Recovery	98.98		98,77	
100% of the target concentration is equivalent to Etodolac-300µg/mL and Dexamethasone-8 µg/mL in acetonitrile: water 50:50 v/v as diluent.					
Accuracy study at 120% target level	Injection Number	Etodolac		Dexamethasone	
		Standard Soln.	Spiked Soln.	Standard Soln.	Spiked Soln.
Dexeto-® tablet dosage form solution at 120% level was spiked with 10% of mixed standard solution of API's	1	2236414	2420796	8174219	8769366
	2	2242501	2409106	8138598	8774954
	3	2226233	2405945	8116684	8793691
	Mean area	**2235049.3**	**2411949.0**	**8143166.9**	**8779337.0**
	Std. Dev	8219.4	7823.0	23709.7	12741.1
	% RSD	**0.4**	**0.3**	**0.3**	**0.1**
	%Recovery		**92**	**94**	
20% of the target concentration is equivalent to Etodolac-360µg/mL and Dexamethasone- 9.6 µg/mL in acetonitrile: water 50:50 v/v as diluent					

Table-4: Evaluation of precision with-in-day and day-to-day analysis

Intra-Day Precision study of 100% standard dilution containing- 300 µg/mL of Etodolac and 8 µg/mL of Dexamethasone.					Inter-Day Precision study of 100% standard dilution containing- 300 µg/mL of Etodolac and 8 µg/mL of Dexamethasone.			
S.No	Dexamethasone		Etodolac		Dexamethasone		Etodolac	
	Ret. time	Peak area	Ret. Time	Peak area	Ret. time	Peak area	Ret. Time	Peak area
1	2.846	6795656	2.099	1834932	2.841	6818152	2.097	1825916
2	2.852	6798398	2.104	1829670	2.852	6801885	2.103	1819983
3	2.842	6872062	2.098	1840002	2.853	6774816	2.104	1838784
4	2.841	6818314	2.097	1847459	2.846	6765719	2.099	1818866
5	2.841	6871487	2.097	1855247	2.851	6754768	2.103	1821671
6	2.852	6841862	2.104	1824900	2.852	6787708	2.104	1836544
Average	2.845	6832963.1	2.099	1838701.6	2.851	6783841	2.101	1826960.5
Std. Dev	0.0052	0.5	0.0033	0.6	0.0048	0.3	0.00295	0.5
% RSD	0.18	34331.0	0.16	11299.5	0.166	23549.5	0.14	8659.2

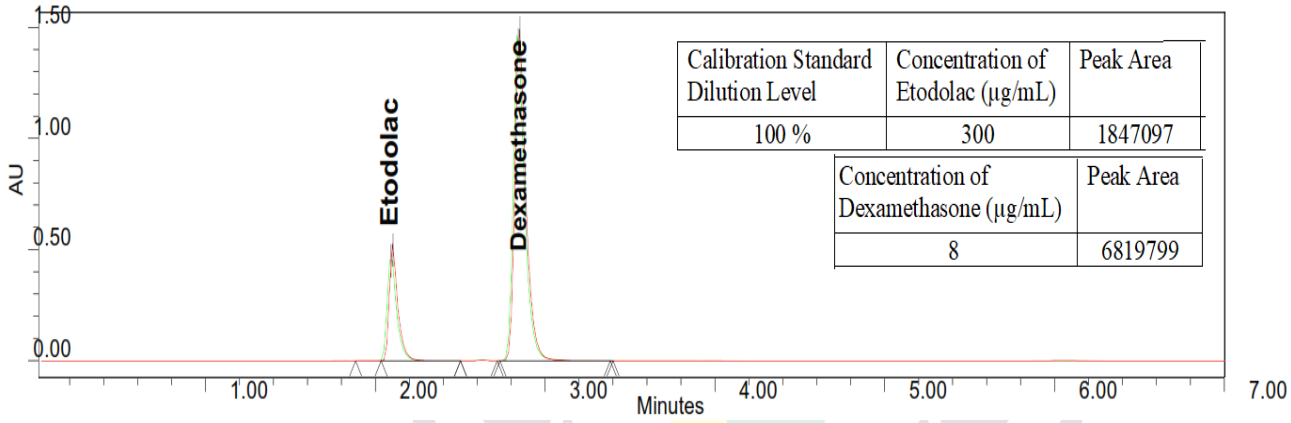
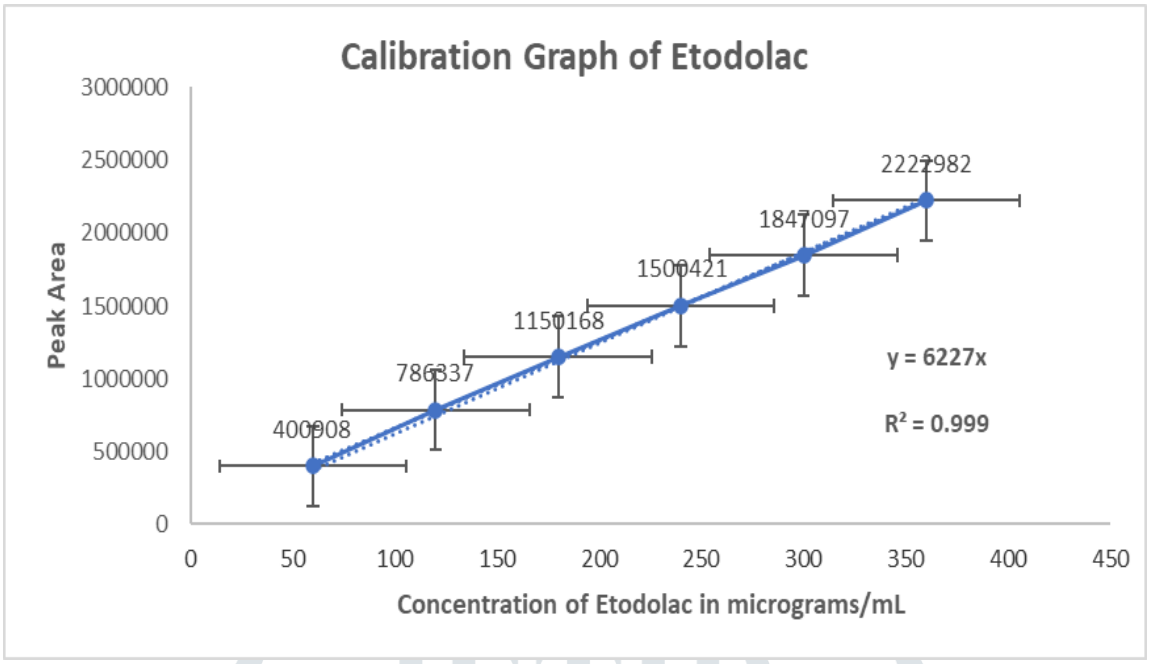


Figure 1: Chromatogram of Etodolac-300µg/mL and Dexamethasone- 8µg/mL analyzed by optimized Isocratic RP-HPLC method



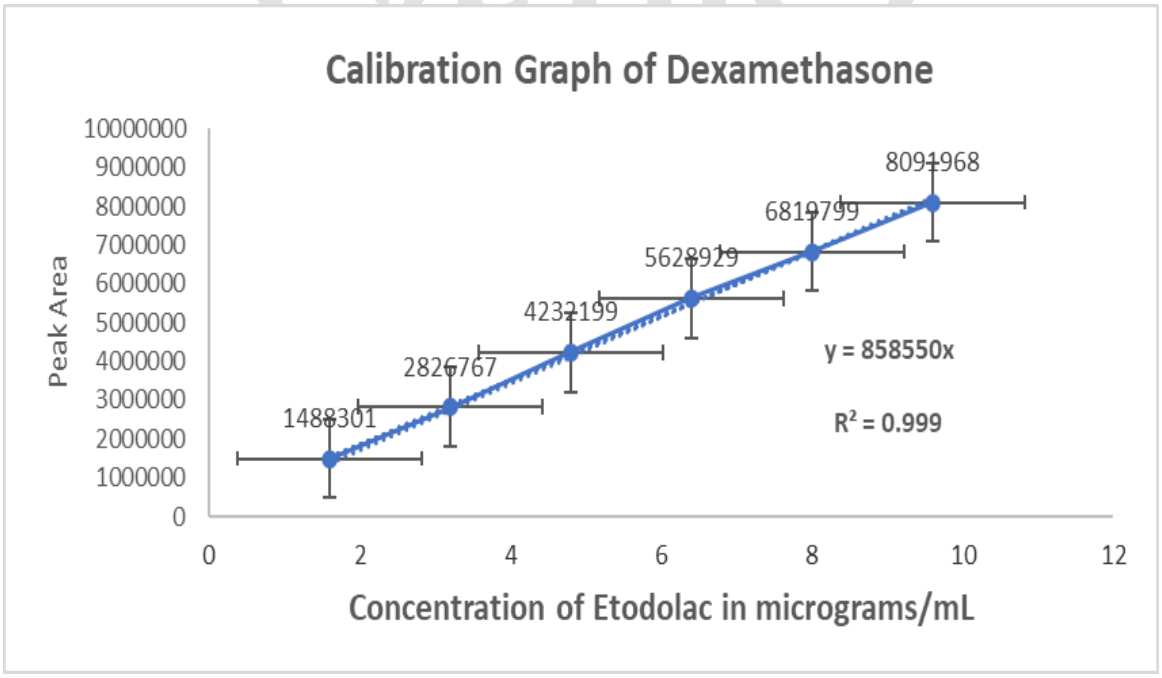


Figure-2: Linearity graphs of Etodolac and Dexamethasone dilutions of standard solutions:

Table 5: The method's Robustness can be tested by changing the chromatographic settings (n=3, 100%w/v Working dilution contains Etodolac-300µg/mL-&-Dexamethasone-8µg/mL)

Table-(a): Robustness study of variation in elution solvents flow rate increase of Etodolac and Dexamethasone:

S. No	Robustness study of Etodolac		Robustness study of Dexamethasone	
	Standard Solution	Sample solution	Standard Solution	Sample solution
1	1723640	1705774	6428738	6375720
2	1723314	1706117	6483222	6374637
3	1725942	1708191	6489911	6376835
Mean	**1724299**	**1706694.0**	**6467290**	**6375730.7**
Std. Dev	1432.5	1307.7	33554.4	1099.0
% RSD	**0.1**	**0.1**	**0.5**	**0.0**
Assay	**99.5**		**99.27**	

Table-(b): Robustness study of variation in elution solvents flow rate decrease of Etodolac and Dexamethasone:

S.No	Robustness study of Etodolac		Robustness study of Dexamethasone	
	Standard Solution	**Sample solution**	**Standard Solution**	**Sample solution**
1	2125004	2071844	7985866	7759345
2	2115500	2092408	7939114	7845217
3	2124965	2096888	7952091	7864386
Mean	**2121823.0**	**2087046.7**	**7959023.7**	**7822982.7**
Std. Dev	5475.9	13355.1	24134.7	55939.0
% RSD	**0.3**	**0.6**	**0.3**	**0.2**
Assay	**99.35**		**99.11**	

Table-(c): Robustness study of variation in stationary phase of Etodolac and Dexamethasone

S.No	Robustness study of Etodolac		Robustness study of Dexamethasone	
	Standard Solution	**Sample solution**	**Standard Solution**	**Sample solution**
1	1899122	1880838	7203273	7022601
2	1895252	1881504	7176251	7070293
3	1897050	1870449	7127300	7017213
Mean	**1897141.3**	**1877597.0**	**7168941.3**	**7036702.3**
Std. Dev	1936.6	6199.3	38510.4	29214.8
% RSD	**0.1**	**0.3**	**0.5**	**0.4**
Assay	**99.50**		**99.40**	

CONCLUSION: In this study, an efficient and commonly available HPLC method for the analysis of Etodolac, and Dexamethasone in pharmaceutical matrices was devised. This method's key advantages are its significantly reduced run times, ease of use, and ease of operation. All these features are critical in operation, especially when analyzing a large number of samples. The validation experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision & accuracy, and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Etodolac, and Dexamethasone.

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