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RESEARCH ARTICLE

STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND ETODOLAC USING RP-HPLC METHOD IN BOTH BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and effective RP-HPLC method had been developed for the estimation of Paracetamol and Etodolac in tablet dosage form, using Inertsil ODS C18 (250 x 4.6 mm, 5m.), mobile phase Acetonitrile and water (60:40), detection wavelength at 269 nm, at flow rate of 1ml/min at retention time 3.206 min for paracetamol, 4.825 min for etodolac. Linearity was obtained in the range of 18.46 µg/ml to 55.38 µg/ml for paracetamol and etodolac. The correlation coefficient was found to be 0.999 for the drugs. The Recovery studies were performed for paracetamol and etodolac in the range of 50% - 150 %. The % Assay for paracetamol is 98.85% and etodolac is 99.81 %. Forced Degradation studies were conducted according to the ICH guidelines and the Drug Product was found to be stable in all conditions. Hence, the method could be successfully applied for routine analysis of Paracetamol and Etodolac in combined dosage form.

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INTRODUCTION

Paracetamol (Figure 1) is chemically N-(4-hydroxyphenyl) acetamide (Siva Rama Krishna *et al.,* 2014). Etodolac is chemically 1,8-Diethyl-1,3,4,9-tetrahydropyrano (3,4-b)indole-1-acetic acid. Both these drugs belong to class of nonsteroidal anti-inflammatory drugs (NSAIDs). A combination of 325mg of Paracetamol and 400 mg of Etodolac is available commercially as tablet. This combination is used as analgesic and antipyretic. Etodolac (Figure 2) is a NSAID and its IUPAC name is (*RS*)-2-(1,8-Diethyl-4, 9-dihydro-3*H*-pyrano [3,4-b] indol-1-yl)acetic acid. This drug is used for the management of mild to moderate pain, fever, and inflammation. It works by reducing the levels of prostaglandins, which are chemicals that are responsible for pain and the fever and tenderness that occur with inflammation. Etodolac blocks the cyclooxygenase enzymes which form prostanoids, resulting in lower concentrations of prostaglandins. As a consequence, inflammation, pain and fever are reduced (Sruthi *et al.,* 2013; Kallur *et al.,* 2013; Mithlesh *et al.,* 2015).

MATERIALS AND METHODS

Chemicals and reagents: Analytically pure sample of paracetamol and etodolac with purities greater than 99% were obtained as gift samples from Chandra labs (Hyderabad, India) and tablet formulation etova-p was procured from Apollo pharmacy, Hyderabad, India with labelled amount 500mg each of paracetamol and etodolac. Acetonitrile (HPLC grade) was obtained from Sigma Aldrich (Hyderabad, India), Water (HPLC grade), 0.45µm Nylon membrane filters were obtained from Spincotech private limited, Hyderabad, India.

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Instrument

HPLC analysis was performed on Shimadzu LC-20AD Prominence Liquid Chromatography comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-Vis detector and Enable C18G reverse phase C18 column (250X4.6 mm, 5 micron particle size). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Lab solutions lite" software. In addition, an electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Selection of wavelength

Initially method development work was started by taking UV-visible spectra from 400-200 nm of paracetamol and etodolac

standard solutions. By observing the spectra of standard solutions λmax 269 nm was chosen as the desired wavelength. The spectrum was shown in Figure No: 5.

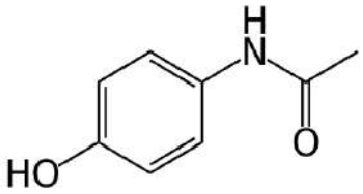

Figure 1. Structure of Paracetamol

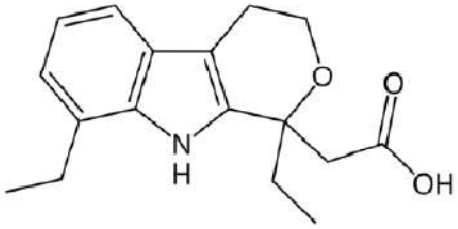

Figure 2. Structure of Etodolac

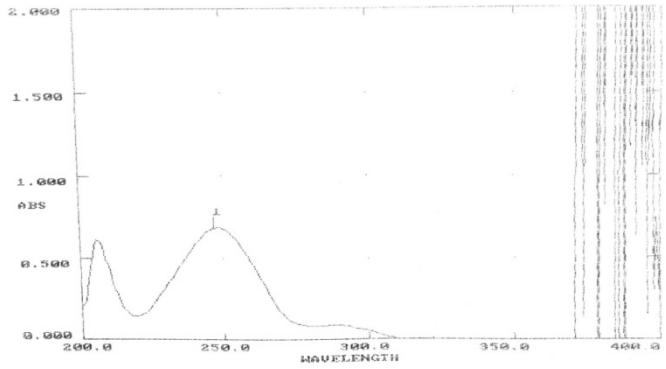

Figure 3. UV spectrum of Paracetamol

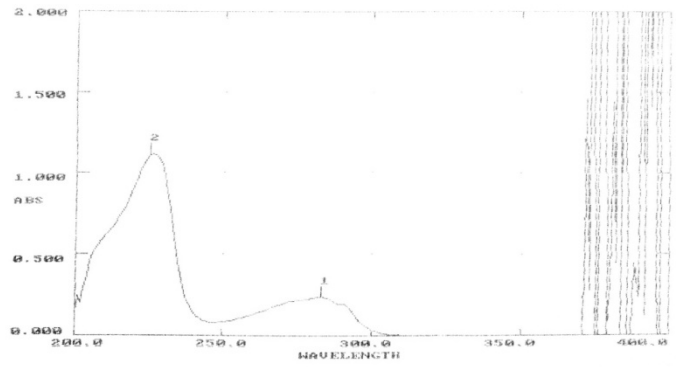

Figure 4. UV spectrum of Etodolac

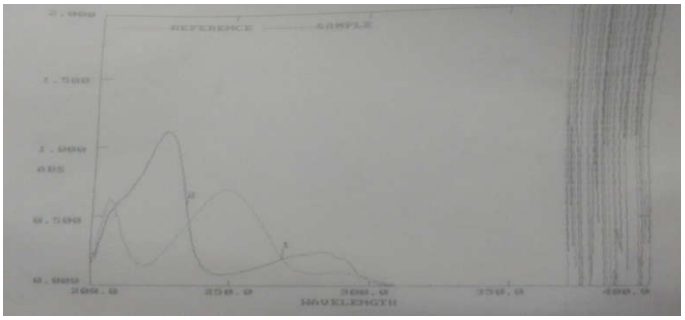

Figure 5. Overlapped UV spectrum of Paracetamol and Etodolac

Chromatographic conditions

The separation of the drugs was achieved on a C18 column reverse phase (4.6 mm X 250 mm, 5 micron particle size). The mobile phase consists of a mixture of Acetonitrile and water as the mobile phase in the ratio 60:40 at a flow rate of 1 ml/minute and the volume injected was 20 µl for every injection .the detection wavelength was set at 269 nm.

Mobile Phase

Prepared a degassed and filtered mixture of Acetonitrile and water were taken in the ratio 60:40 v/v.Peaks of paracetamol and etodolac were well resolved with the solvent system of Acetonitrile:water in the ratio of 60:40 v/v.

Preparation of Stock and Working Standard Solution

10mg of Paracetamol and Etodolacwere accurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml of solvent (mobile phase)and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 3.7ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 36.92µg/ml, treated as working standard, 100% target concentration for which UV spectrum was recorded (Figure 5).

Preparation of Stock and Working Sample Solution

Take average weight of one tablet and crush in a mortar by using pestle and weight 99.8 mg equivalent weight ofetova-p sample into a 100ml clean dry volumetric flask and add about 80ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent (stock 2) from this stock-2 pipette out 3.7 ml into 10 ml clean dry volumetric flask and add diluent upto the mark to get target concentration of 36.92µg/ml of concentration.

RESULTS AND DISCUSSION

Method Development

RP-HPLC isocratic stability indicating assay method was developed keeping in mind the system suitability parameters i.e. Asymmetric factor (A),number of theoretical plates (N), runtime, separation of drug peak from the forced degradants, detection of drug peak along with significant impurities and majority of impurities. In order to test the applicability of the developed method to a commercial formulation, etova-p was chromatographed at working concentration (36.92µg/ml) and it is shown in Figure 8. The samplepeak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 90 and 105%, which is the standard level in any pharmaceutical quality control.

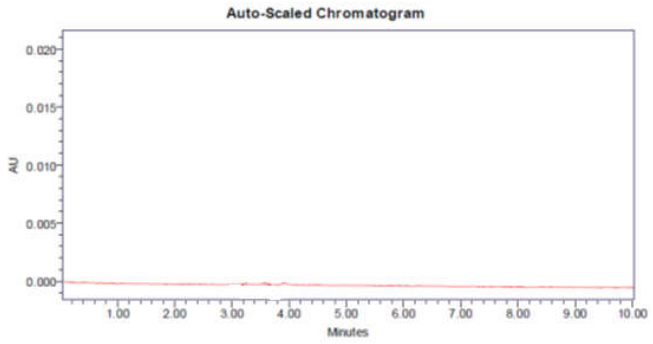

Fig. 6. Typical chromatogram of the blank

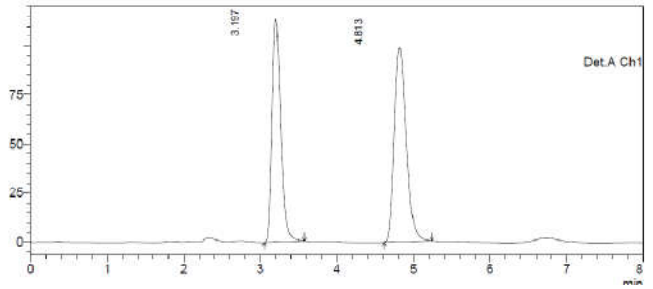

Fig. 7. Typical chromatogram of the standard

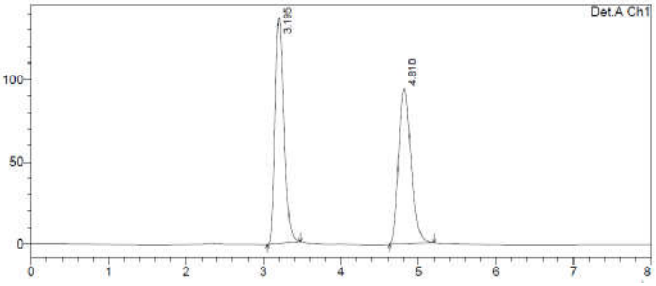

Fig. 8. Typical chromatogram of the control formulation

Method Validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, and sensitivity.

Specificity

Blank, standard drug solution and sample chromatogram revealed that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drug as blank had no peak at the retention time of paracetamol and etodolac. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System Precision

Six replicate injections of the standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 1.

Method Precision

Method precision was determined by performing assay of sample under the tests of repeatability at working concentration.

Repeatability (Intraday precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 2).

Table 1. System Precision Results

	Paracetamol		Etodolac	
S.No.	Rt	Area	Rt	Area
1	3.197	360763	4.813	429725
2	3.198	357025	4.816	429021
3	3.203	361028	4.820	425498.000
4	3.199	359175	4.814	427583.000
5	3.192	356969	4.806	425614
6	3.191	352818	4.807	414863.000
avg	3.1967	357963	4.813	425384.000
stdev	0.0045	3067.314	0.005	4960.930
%RSD	0.14	0.86	0.11	1.17

Table 2. Intraday Precision Results

	PARACETAMOL		ETODOLAC	
S.No.	Rt	Area	Rt	Area
1	3.627	2646.619	5.047	2433.63
2	3.623	2647.154	5.040	2421.25
3	3.630	2647.393	5.047	2427.526
4	3.630	2653.772	5.043	2433.302
5	3.623	2658.543	5.033	2437.33
6	3.630	2657.198	5.043	2428.506
avg	3.6272	2651.780	5.042	2430.256
stdev	0.0034	5.410	0.005	5.699
%RSD	0.09	0.20	0.10	0.23

Intermediate Precision (Ruggedness / Inter day precision)

The sample solution from the same homogeneous mixture at working concentration on a different day by a different analyst, showed % RSD less than 2 for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged (Table 3).

Table 3. Intermediate Precision

		analyst 1			
		Paracetamol	etodolac		Result
1	Standard	1102591	1017647	paracetamol	98.34735
2	Sample	1084369	1014451	etodolac	99.68594
		analyst 2			
		Paracetamol	etodolac		Result
1	Standard	1122641	1018626	paracetamol	100.881
2	Sample	1132532	1019236	etodolac	100.0599

Linearity

Standard solutions of paracetamol and etodolacat different concentrations level (50%, 75%, 100%, 125% and 150%) were

prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area. The results show an excellent linear correlation between peak area and concentration level of drug within the concentration range (18.46-55.38µg/ml) for the drug and the results are given in Table 4 and Figure 9 & 10**.** The correlation coefficient of paracetamol is 0.999andetodolac is 0.999. Thus hence the method is said to be linear in the range of 18.46-55.38 µg/ml.

Table 4.

S.No	Concentration	Dilution (µg/ml)	Area of paracetamol	Area of etodolac
1	50%	18.46	491509	448758
2	75%	27.69	812948	752425
3	100%	36.92	1099557	1049085
4	125%	46.15	1449987	1390158
5	150%	55.38	1778739	1701530
regression coefficient			0.999088155	0.999461581
slope(m)			1284599.6	1257310.8
intercept (c)			-158051.6	-188919.6
regression equation			Y=1284599X+158051	Y=1257310X+188919

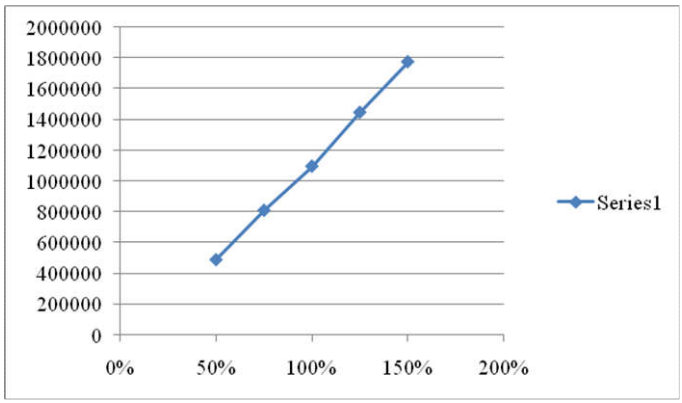

Fig. 9. Linearity Graph of PARACETAMOL

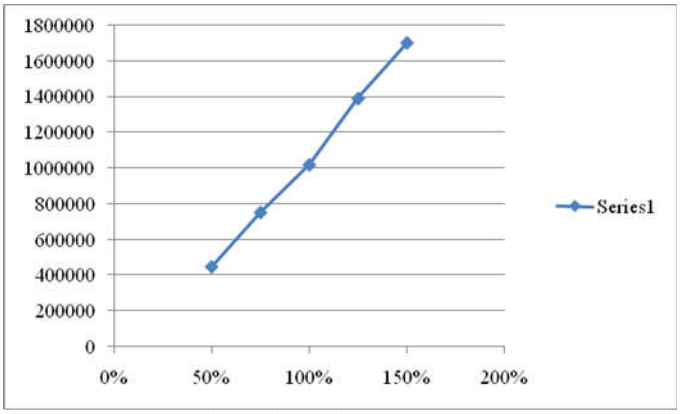

Fig. 10. Linearity Graph of ETODOLAC

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 5 & 6. The accepted limits of recovery are 90%-110% for the process of determining recovery of the standard from the formulation at three different levels of 50%, 100% and 150%. All observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 5. Recovery Studies Results of Paracetamol

ACCURACY OF PARACETAMOL				
S.No	%LEVEL	Sample area	% of recovery	% of mean recovery
1	50%-01	1096527	96.63176318	
2	50%-02	1186427	104.554227	100.3335249
3	50%-03	1132644	99.81458438	
1	100%-01	1294267	102.6518928	
2	100%-02	1243207	98.60218304	98.86811874
3	100%-03	1202206	95.35028041	
1	150%-01	1496458	105.5006398	
2	150%-02	1407342	99.2179409	101.3352979
3	150%-03	1408326	99.28731313	

Table 6. Recovery Studies Results of Etodolac

ACCURACY OF ETODOLAC				
S.no	% level	Sample area	% recovery	% Of mean recovery
1	50%-01	156550	73.60408478	102.83
2	50%-02	158072	74.31967352	
3	50%-03	157527	74.06343445	
1	100%-01	421600	99.11045079	
2	100%-02	423321	99.51502642	99.36
3	100%-03	420470	98.84480846	
1	150%-01	672567	94.86492205	
2	150%-02	671439	94.70581874	
3	150%-03	673068	94.93558761	99.103

Sensitivity

The sensitivity of measurement of PARACETAMOL and ETODOLAC by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of response of calibration plot and S is the slope of the corresponding calibration plot. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.001924ng/mland0.00583ng/ml respectively for PARACETAMOL and 0.001109ng/ml and 0.003361ng/ml for ETODOLAC respectively.

FORCED DEGRADATION STUDIES

SAMPLE PREPARATIONS TO PERFORM FORCED DEGRADATION STUDIES

Acid degradation

Take tablet powder and crush in a mortar by using pestle and weigh one tenth equivalent weight of (99.8 mg) etova-p sample into a 100mL clean dry volumetric flask and add about 3mL of 0.5N HCl and kept a side for 3hours and add 3mL of 0.5N NaOH solution to neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 1ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent from this again pipette out 3.7 ml into 10 ml volumetric flask having diluent make up to the mark with the diluent and degassed in a digital ultrasonicator for 10 minutes.

Alkaline degradation

Take tablet powder and crush in a mortar by using pestle and weight 99.8 mg equivalent weight of etova-p sample into a 100mL clean dry volumetric flask and add about 3mL of 0.5N NaOH and kept a side for 3hours and add 3mL of 0.5N Hcl solution to neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely.

Further pipette 1ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent from this again pipette out 3.7 ml into 10 ml volumetric flask having diluent make up to the mark with the diluent and degassed in a digital ultrasonicator for 10 minutes.

Peroxide degradation

Take tablet powder and crush in a mortar by using pestle and weigh 99.8 mg equivalent weight of etova-p sample into a 100mL clean dry volumetric flask and add about 10mL of 3% Hydrogen peroxide solution and kept a side for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 1ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent from this again pipette out 3.7 ml into 10 ml volumetric flask having diluent make up to the mark with the diluent and degassed in a digital ultrasonicator for 10 minutes.

Thermal degradation

Take tablet powder and crush in a mortar by using pestle and weight 99.8 mg equivalent weight of etova-psample into a 100mL clean dry volumetric flask and expose to heat at 70ºc for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 1ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent from this again pipette out 3.7 ml into 10 ml volumetric flask having diluent make up to the mark with the diluent and degassed in a digital ultrasonicator for 10 minutes

Photolytic degradation

Take tablet powder crush in a mortar by using pestle and weight 99.8 mg equivalent weight of etova-psample into a 100mL clean dry volumetric flask and expose to ultraviolet rays for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 1ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent from this again pipette out 3.7 ml into 10 ml volumetric flask having diluent make up to the mark with the diluent and degassed in a digital ultrasonicator for 10 minutes

Table 6. Results for degradation studies of Paracetamol

S. No	Condition	Final area	Initial area		%degradation
1	ACIDIC	2658.543	2651.779833	-6.76317	-0.255042541
2	BASIC	2648.553	2651.779833	3.226833	0.121685567
3	HEAT	2648.553	2651.779833	3.226833	0.121685567
4	UV	2660.236	2651.779833	-8.45617	-0.318886454
5	PEROXIDE	2653.772	2651.779833	-1.99217	-0.075125644

Table 7. Results for degradation studies of Etodolac

FORCED DEGRADATION-ETODOLAC					
S. No	Condition	Final area	Initial area		% degradation
1	ACIDIC	2437.326	2430.256	-7.07	-0.290915854
2	BASIC	2428.364	2430.256	1.892	0.077851881
3	HEAT	2428.364	2430.256	1.892	0.077851881
4	UV	2436.532	2430.256	-6.276	-0.258244399
5	PEROXIDE	2433.302	2430.256	-3.046	-0.12533659

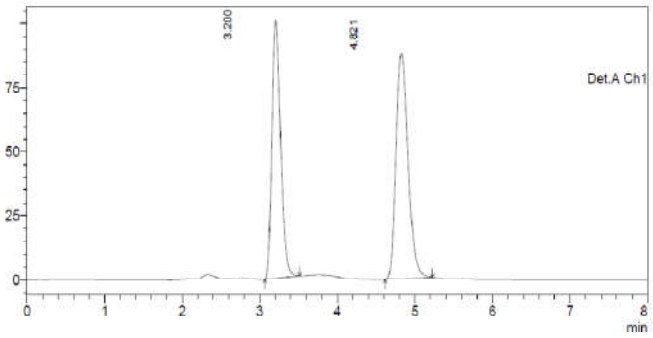

Figure 11.Chromatogram showing acidic degradation.

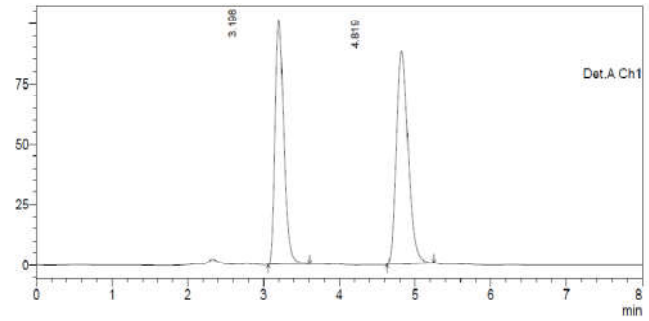

Figure 12. Chromatogram showing basic degradation.

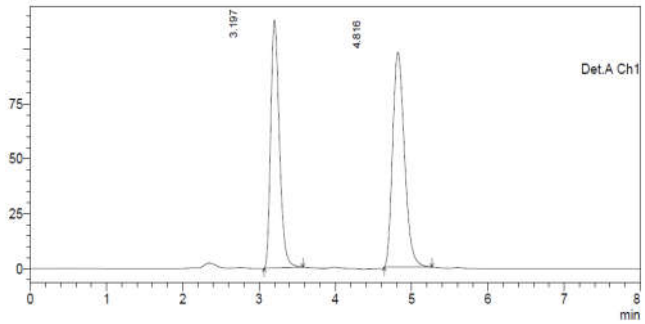

Figure 13. Chromatogram showing peroxide degradation.

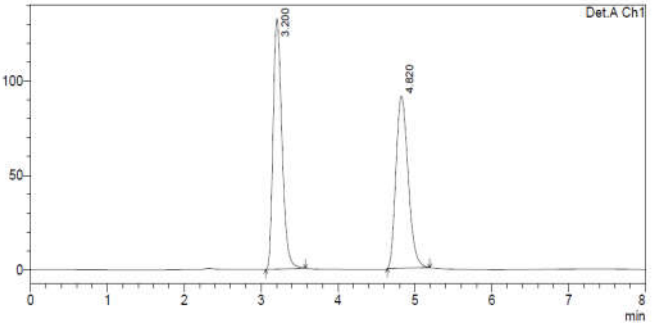

Figure 14. Chromatogram showing sunlight degradation.

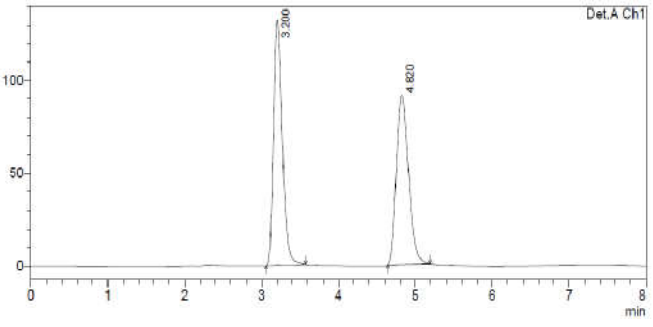

Figure 15. Chromatogram showinguv degradation.

Conclusion

A reverse phase HPLC isocratic stability indicating assay method has been developed and validated as per ICH guidelines for the quantitative estimation of PARACETAMOL and ETODOLAC in etova-p tablets. Forced degradants were separated from the drug peak using the optimized method. Intraday is exemplified by relative standard deviation of 0.2% &0.23%. A good linear relationship was observed for the drug between concentration ranges of 18.46 and 55.38 µg/ml. Accuracy studies revealed that mean recoveries were between 90 and 110%, an indicative of accurate method. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.001924ng/mland0.00583ng/ml respectively for PARACETAMOL and 0.001109ng/ml and 0.003361ng/ml for ETODOLAC respectively. Accordingly it can be concluded that the developed reverse phase isocratic HPLC stability indicating assay method is sensitive, accurate, precise and linear and therefore the method can be used for the routine analysis.

REFERENCES

Balan, P., CarolinNimila, I., Lakshmi Prasanna, M., Vanaja Rani, M., Rajasekar, S. 2011. Simultaneous estimation of Etodolac and Paracetamol by UV spectrophotometric method in tablet formulation. *Journal of Pharmacy Research,* 4(6):1663-1665.

International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1). 2005.

Jadavalpa, V., GohelBhavika, A., SondagarMital, M., Patel Bhavna, A., Parmar Shraddha, J. 2013. Method development and validation for the simultaneous estimation of Paracetamol and Etodolac by Derivative UV spectroscopic method. *International Journal of Pharm Tech Research,* 5(3):1155- 1160.

Kallur, H.J., Prabhudev, S.M., Bagal, V.A., Kishore Singh, C. 2013. Simultaneous estimation of Etodolac and Paracetamol in bulk drug dosage form by RP-HPLC. *International Journal of Advances in Pharmaceutical Analysis,* 3(3):56-67.

Mithlesh, R., Hinna, H, Manjeet, A., Rakesh Kumar, K. 2015. Development and validation of a stability indicating Reverse Phase HPLC Method for simultaneous determination of Etodolac and Paracetamol in its tablet dosage formulation. *International Journal of Innovative Research in Science, Engineering and Technology,* 4(1):19075-19081.

Shailaja, B., Afreen, M., Vinay Kumar, T., Ashok Kumar, A. 2015. UV assay method development and validation for simultaneous estimation of Etodolac and Paracetamol in tablets by simultaneous equation method. *Indo Am J Pharm Sci.,* 2 (11), 1468-1473.

Siva Rama Krishna, V., Sateesh, B., Ravi, N.T. 2014. RP-HPLC method development and validation of Etodolac and Paracetamol in tablet dosage form. *International Journal of Pharm Tech Research,* 6(2):775-782.

Sruthi, A., Thanuja, N., Saisamhita, M., Sudheerkumar, D., Sreekanth, G. 2013. A simple RP-HPLC method for simultaneous estimation of Paracetamol and Etodolac in tablet dosage form Indo American Journal of Pharm Research, 3(5):3742-3751.

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