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RESEARCH ARTICLE

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF AMLODIPINE BESYLATE AND CELECOXIB IN SYNTHETIC MIXTURE.

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Abstract

A simple, rapid, economical, precise and accurate HPLC method for simultaneous estimation of Celecoxib and Amlodipine Besylate in their combined dosage form has been developed.

A Stability indicating reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Celecoxib and Amlodipine Besylate in their combined dosage form. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (Potassium Phosphate, pH 4.5): Methanol (85:15) at a flow rate of 1 ml/min. Detection was carried out at 240 nm. Retention time 4.080 min and 5.343 for Celecoxiband AmlodipineBesylaterespectively.Themethodhasbeenvalidatedforlinearity, accuracy and precision. Linearity observed for Celecoxib 20-60 µg/ml and for Amlodipine Besylate 1-3 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneousestimationofCelecoxibandAmlodipineBesylateintheircombineddosageform. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosageform.

The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in Thermal degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form.

Introduction:-

Amlodipine besylate(AML) 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)- 6-methyl-1,4-dihydropyridine-3,5-dicarboxylate-benzene sulfonic acid is potent dihydro Calcium channel blocker Antihypertensive drug.Various analytical methods have been reported for the assay of AML alone or in combination with other Pharmaceutical Formulations.

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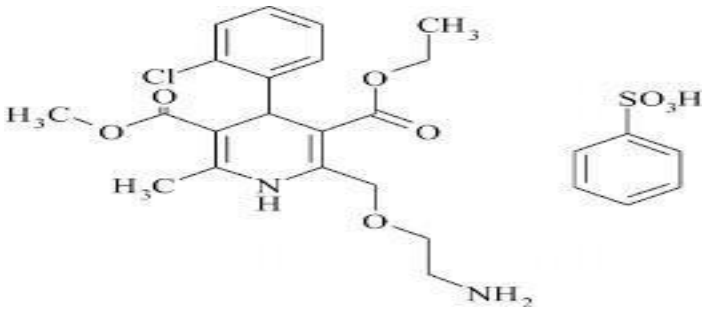

Fig 1:-Structure of Amlodipine besylate

Celecoxib (CEL)4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide is Nonsteroidal anti-inflammatory drug.Various analytical methods have been reported for the assay of CEL alone or in combination with other PharmaceuticalFormulations.

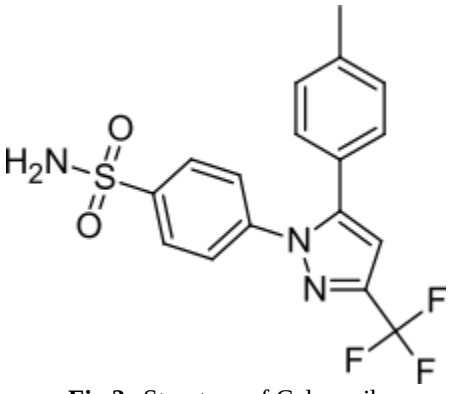

Fig 2:-Structure of Celecoxib

Both Drugs are official in IP and BP. Literature survey revealed that there are several methodswerereportedfortheestimationofAMLandCELindividually.Asnoanalytical method is available for their simultaneous estimation, however, it is essential to develop a suitableanalyticalmethodforsimultaneousestimationofAMLandCELinbulkand in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivityandspecificity.Wehavedevelopedasimple,precise,accurateandspecificRP- HPLC method for the simultaneous estimation of AML and CEL in bulk and in pharmaceutical dosage forms. Because analytical methods must be validatebefore use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference in Harmonization (ICH)[10] guidelines,byassessingitsselectivity, linearity,accuracy,andprecision,limit ofdetection and limitofquantification.

Materials And Methods:-

Reagents and Chemical

Amlodipine besylate and Celecoxib were procured from Prudence pharmachem Ankles war, Gujarat, India. The market. HPLC grade reagents methanol, acetonitrile (Merck specialists pvt, Ltd Mumbai) were used for study. All the reagent prepared by carbon dioxide free water and whereas the sample solution prepared in carbon dioxide free water double Distilled water for HPLC Purpose.

Apparatus and chromatographic conditions

HPLC method development and validation was done on a HPLC instrument(LC 20AT) PDA detector, Stationary Phase used was C18 Hypersil BDS, 250 x 4.6 mm, 5µ column particle size and mobile phase consisting of Buffer, (phosphate Buffer (pH 4.5): Methanol (85:15)was used. The flow rate was 1.0 ml/min and the effluents were monitored at 240 nm. The mobile phase was filtered through nylon 0.45 µm membrane filter (Millipore Pvt., Ltd, Bangalore, India). Injection volume was 20 µL. All weighing were done on analytical balance.

Preparation of mobile phase

The mobile phase was prepared with accurately weighed 6.8 gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask added about 900ml of milli-Q water and degas to sonicated and finally make up the volume with water then pH adjusted to 4.5 with dil.Orthophosphoricacidsolution.Bufferandmethanoltakenintheratio85:15.Themobile phase was degassed for 15 minutes beforeuse.

Preparation of Standard solutions

Amlodipine Besylate standard stock solution: (2 ppm)

A 20 mg of Amlodipine Besylate was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with diluent. Take 10ml from this Solution and transfer to 100ml Volumetric flask and Volume was made up with the diluent.Take 1ml from this solution and transferred int0 10 ml volumetric flask and volume made up with the diluent.

Celecoxib standard stock solution: (40 ppm)

A 40 mg of Celecoxib was weighed and transferred to a 100 mL volumetric flask and Volume made up with the diluent.Take 1 ml from this solution and transferred int0 10 ml volumetric flask and volume made up with the diluent.

Preparation of Sample solutions

Take weigh of powder sample equivalent to 20 mg AML and 400 mg CEL and transferred into 100 ml volumetric flask. Sonicated the solution for 30 min with few ml of diluent. Take 10 ml from above solution and transferred into 100 ml vol.flask make up to mark with diluent.

Result And Discussion:-

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

To evaluate the linearity of the method, six different dilutions were made from the standard stock solutions in the working range of 1-3 ppm and 20-60 ppm for Amlodipine besylate and Celecoxib respectivey.

In order to determine the accuracy of the method, three different concentrations (80%, 100% and 120%) of tablet formulation were used and their recovery was calculated. Regarding the determination of the precision (repeatability) five replicate injections of the working standard Amlodipine besylate and Celecoxib were injected and the relative standard deviation (RSD) of the peak areas were calculated for the replicate injections. To determine the LOD and LOQ, serial dilutions of the combination were made from the standard stock solution the signal from the samples was compared with those of blank samples.

System suitability

As per USP-24, system suitability tests were carried out on freshly prepared standard stocksolutionofAmlodipinebesylateandCelecoxibunderoptimizedchromatographic condition and parameters were studied to evaluate the suitability of the system.Results are shownTable.1

Table.1 System suitability testing

Parameters	Amlodipine Besylate	Celecoxib
Retention time (min)	5.343	4.080
Theoretical plate	3369	7180
Tailing factor	1.264	1.385
Resolution	4.505	

Linearity and Range

The linearity for Amlodipine Besylate and Celecoxib were assessed by analysis of combined standard solution in range of 1-3 µg/ml and 20-60 µg/ml respectively into 100 ml volumetric flaskandmakeupwithmobilephase.Correlationco-efficientfor calibrationcurveAmlodipine Besylate and Celecoxib was found to be 0.999 and 0.999respectively.

Table 2:-Linearity data for Amlodipine Besylate

Sr. No	Concentration (µg/ml)	Area
1	1	102.27
2	1.5	147.49
3	2	202.41
4	2.5	251.36
5	3	306.01

Table 3:-Linearity data for Celecoxib

Sr. No	Concentration (µg/ml)	Area
1	20	510.871
2	30	735.164
3	40	1008.459
4	50	1251.952
5	60	1507.440

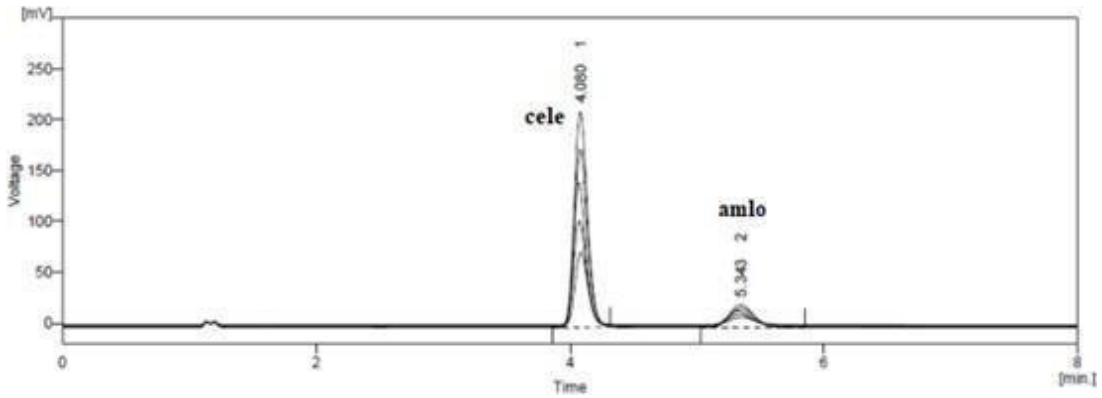

Fig 3:-Overlay chromatogram of different concentrations of mixtures of Amlodipine Besylate and Celecoxib

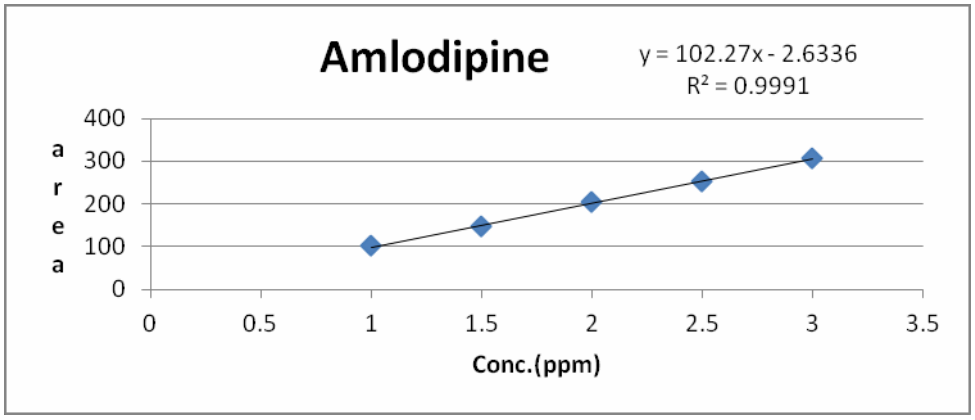

Fig 4:-Calibration Curve of Amlodipine Besylate (1-3 ppm)

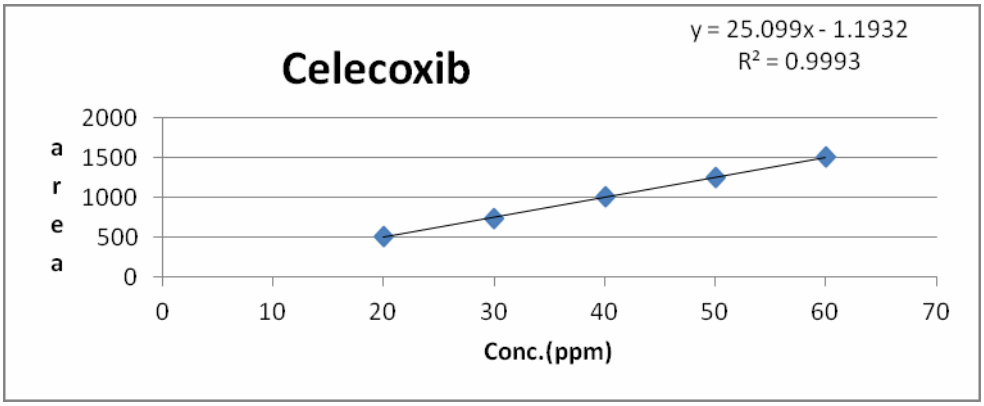

Fig 5:-Calibration Curve of Celecoxib (20-60 ppm)

Accuracy

Accuracy was determined by recovery studies of Amlodipine besylate and Celecoxib, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 4 & 5. The study was done at three different concentration levels.

Table 4:-Recovery data for Amlodipine Besylate

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1	0.8	0.807	100.823	100.383 ± 0.973
2		1	0.8	0.808	101.058	
3		1	0.8	0.794	99.268	
4	100 %	1	1	1.001	100.122	100.937 ± 1.205
5		1	1	1.023	102.321	
6		1	1	1.004	100.367	
7	120 %	1	1.2	1.213	101.048	100.388 ± 0.594
8		1	1.2	1.203	100.219	
9		1	1.2	1.199	99.896	

Table 5:-Recovery data for Celecoxib

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	20	16	16.194	101.214	100.312 ± 1.191
2		20	16	16.122	100.762	
3		20	16	15.834	98.962	
4	100 %	20	20	19.963	99.814	100.889 ± 0.948
5		20	20	20.249	101.247	
6		20	20	20.321	101.606	
7	120 %	20	24	24.179	100.747	101.296 ± 0.663
8		20	24	24.488	102.032	
9		20	24	24.266	101.109	

Precision

The precision (repeatability) of an analytical method refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the %RSD. The precision study (**Table 6 & 7**) showed thatmethod hasagoodreproducibilitywhichwasapprovedbythe analysisoffivereplicate injections of the working standardsolutions.

Table 6:-Repeatability data for Amlodipine Besylate

Amlodipine Besylate				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	2	203.033 202.648 200.811 202.615 202.200 204.242	202.592 ±1.118	0.552

Table 7:-Repeatability data for Celecoxib

Celecoxib				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	40	1011.478 1009.442 996.977 1009.389 1007.360 1017.485	1008.689±6.710	0.665

Intraday Precision

Standard solution containing (20,40,60 µg/ml) of Celecoxib and (1,2,3 µg/ml) of Amlodipine Besylate were analyzed three times on the same day and % R.S.D was calculated.

Table 8:-Intraday precision data for estimation of Amlodipine Besylate

	Amlodipine Besylate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	101.769 ± 0.177	0.174
2	2	203.844 ± 1.446	0.709
3	3	301.321 ± 3.841	1.274

Table 9:-Intraday precision data for estimation of Celecoxib

	Celecoxib		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	20	507.460 ± 2.414	0.476
2	40	1020.596 ± 4.406	0.432
3	60	1510.421± 4.710	0.312

Interday precision

Standard solution containing (20,40 60 µg/ml) of Celecoxib and (1,2,3 µg/ml) of Amlodipine Besylate were analysed three times on the different day and % R.S.D was calculated.

Table 10:-Interday precision data for estimation of Amlodipine Besylate

	Amlodipine Besylate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1	101.345 ± 1.913	1.888
2	2	199.053± 2.104	1.057
3	3	304.791 ± 0.914	0.300

Table 11:-Interday precision data for estimation of Celecoxib

	Celecoxib		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	20	509.291 ± 4.371	0.858
2	40	990.636± 12.191	1.231
3	60	1516.091± 7.122	0.470

Limit of detection and Limit of Quantification

Thedetectionlimitor LODisthelowest amountofanalyteinasamplethatcanbedetectedItmay be expressed as a concentration that gives a signal to noise ratio of approximately 3:1. While the Quantification limit or LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy with a signal to noise ratio of approximately 10:1 can be takenasLOQofthe.Our methodshowedthe(LOD)forAmlodipineandCelecoxibwere foundto be0.092µg/ml and1.557 µg/mlrespectivelyandTheLOQvaluesforAmlodipineandCelecoxib were found 0.278 µg/ml and 4.717 µg/mlrespectively.

Robustness

The robustness of the proposed method was evaluated by slight modification in the mobile phase compostion, Flow rate and pH values of the mobile phase . During these studies it was found that there was not much change retention time, area andsymmetry ofpeak.Thedeveloped methodwasusedfortheassayofcommerciallyavailabletablets and six replicate determinations were performed. The interference of excipients was studied by comparing the chromatography of standards and formulations. The same shape and retention times of peaks showed that there was no interference from excipients.

Force degradation study

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was carried out by exposing of samples to stability condition 1 N HCl at 60 ^{0}C 90 min, 0.5 N NaOH at60 ^{0}C 90 min ,3% H2O2 at 25 ^{0}C for 6 hrs , 110 ^{0}C for 3 hrs and UV 18 hrs. They were then analysed againstcontrol.

Drugs	**Retention time(min)**	**Area (mV.s)**
Amlodipine Besylate	5.033	188.104
Celecoxib	3.753	916.253

Table 12:-Amlodipine Besylate % Degradation

Amlodipine Besylate				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	150.515	19.983	153.131	18.592
Base	155.049	17.573	145.500	22.649
Thermal	158.013	15.997	163.108	13.288
Oxidation	152.928	18.700	149.068	20.752

Table 13:-Celecoxib % Degradation

Photo	152.282	19.044	153.976	18.143
Celecoxib				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	798.943	12.803	787.870	14.012
Base	662.278	27.719	682.565	25.505
Thermal	638.708	30.291	619.163	32.424
Oxidation	749.528	18.196	738.145	19.439
Photo	815.743	10.970	806.221	12.009

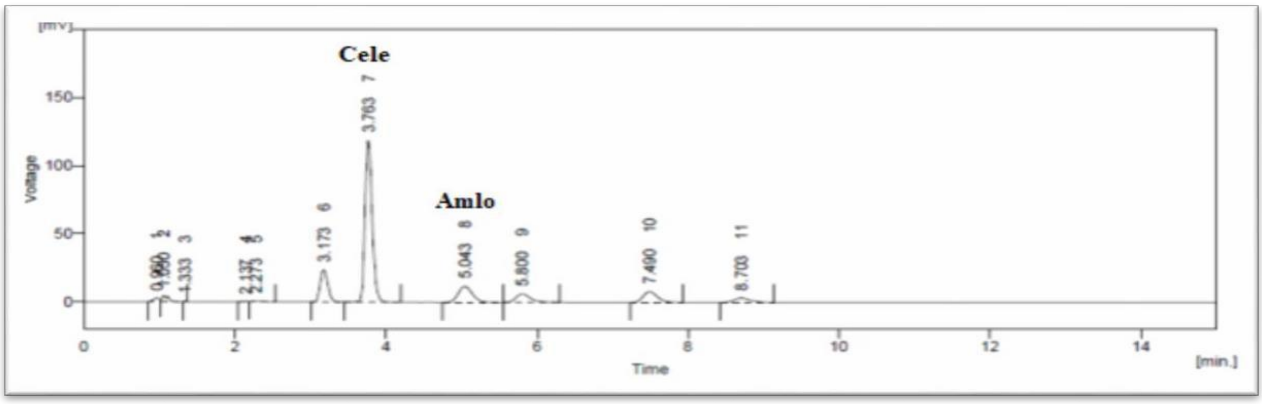

Fig 6:-Amlodipine besylate and Celecoxib Acid Degradation Sample at 60℃ for 90 min

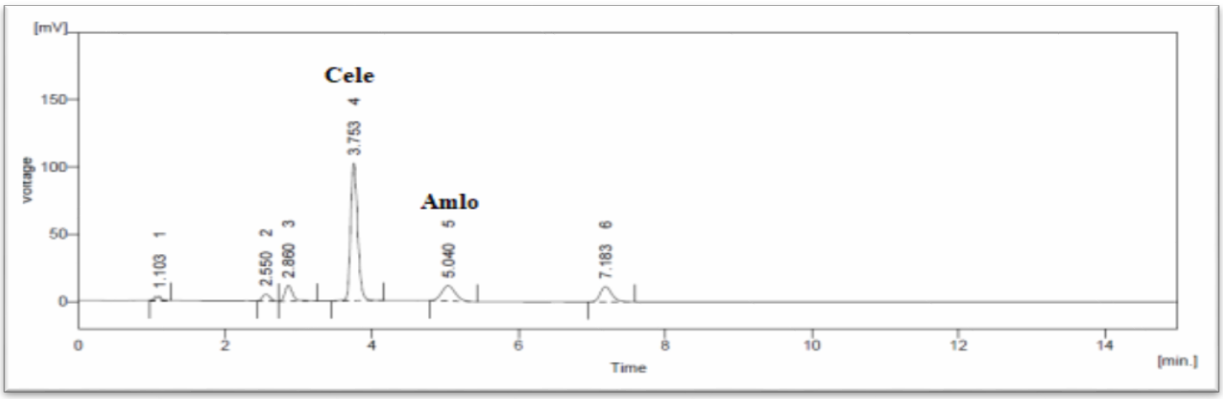

Fig 7:-Amlodipine Besylate and Celecoxib Base Degradation Sample at 60℃ for 90 min

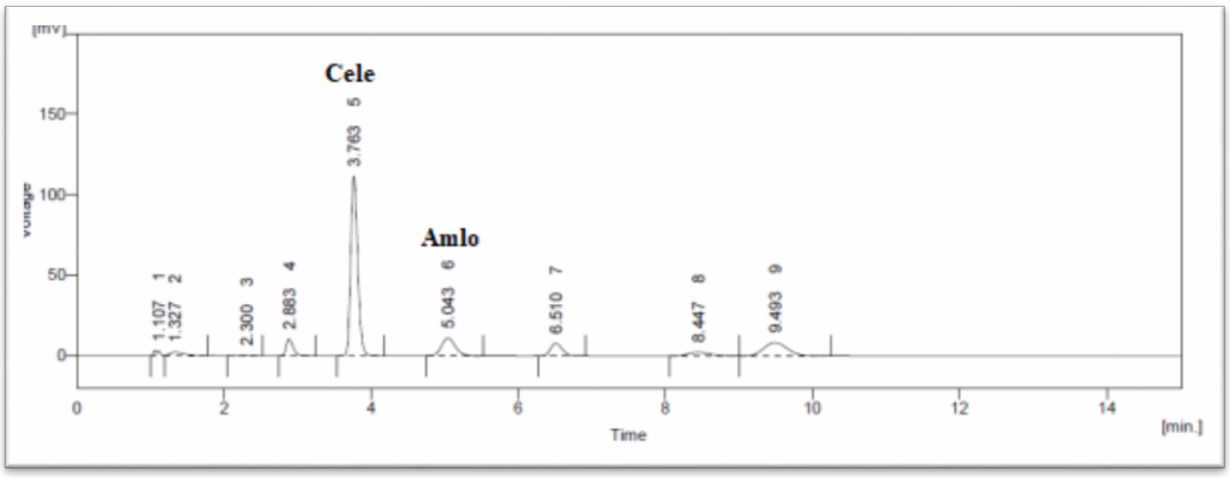

Fig 8:-Amlodipine Besylate and Celecoxib Oxidation Degradation sample in 3% H2O2 at Room temperature for 6 hrs

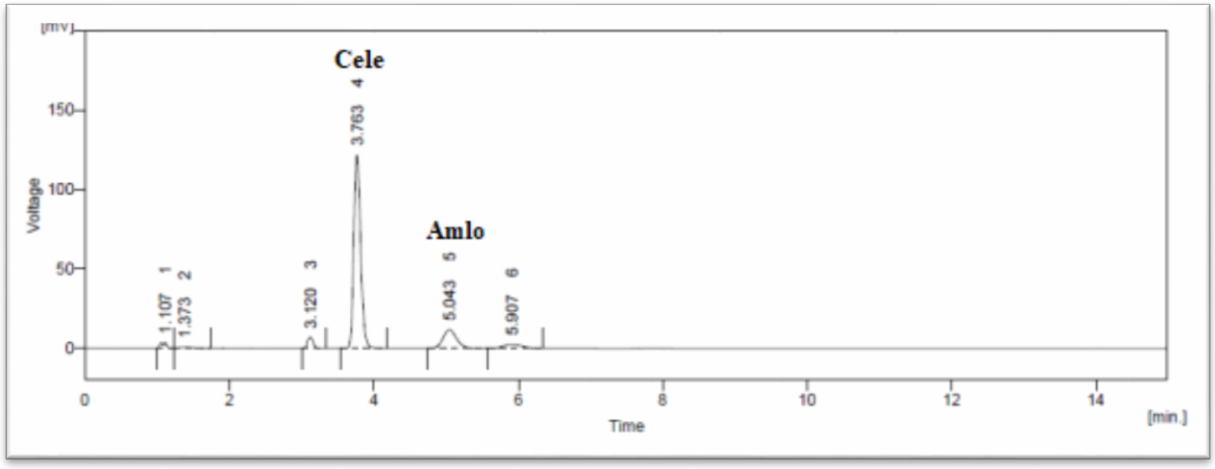

Fig 9:-Amlodipine Besylate and Celecoxib Photo Degradation sample at 254nm UV light (18hrs)

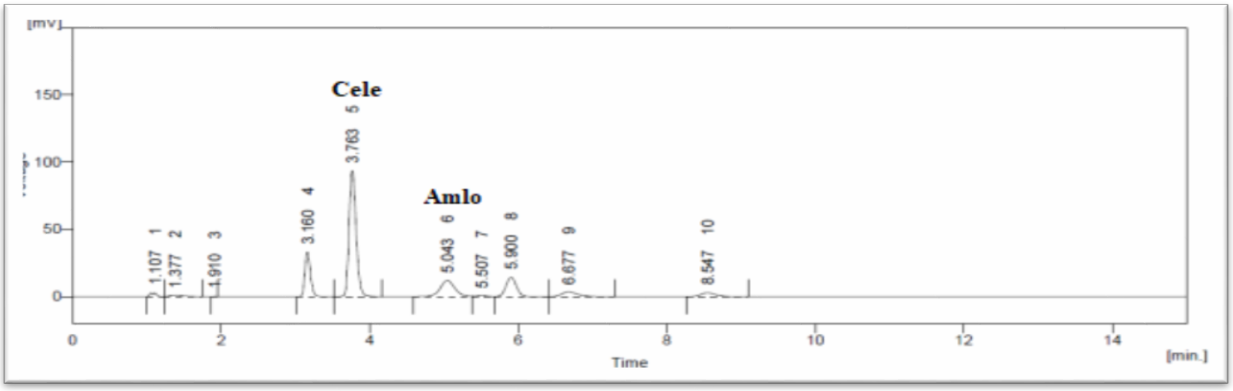

Fig. 10:-Amlodipine Besylate and Celecoxib Thermal Degradation sample at 110^0C for 3 hrs

Conclusion:-

A simple, specific, accurate and precise Stability indicating RP-HPLC method has been developed and validated as per ICH guideline for Simultaneous Estimation of Celecoxib and Amlodipine Besylate in their combined dosage form.Validation parameters like Linearity, Accuracy, Precision, Robustness, Systemsuitability, Specificityweretested. Observationofall these parameters leads to the point that developed Stability indicating RP-HPLC method is linear, accurate, precise, specific and robust. It can be successfully adopted for routine quality control analysis of Celecoxib and Amlodipine Besylate in Combined dosage formwithout any interference from common excipients and impurity.This method can now transfer to utilize for routine laboratory analysis and assay of Celecoxib and Amlodipine Besylate in their combined dosageform.

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