DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING ASSAY OF ALPHA LIPOIC ACID AND ENZOGENOL BY UPLC AND ITS DEGRADATION

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Abstract: A validated stability-indicating UPLC method for Alpha Lipoic Acid and Enzogenol was developed by separating its degradation products on a RP18 (150x4.6mm, 2.1µm) Waters X-Terra column using water and acetonitrile in simple Isocratic at a flow rate 1.0 ml/min. The column effluents were monitored by a photodiode array detector set at 228 nm. The method was validated in terms of specificity, linearity, accuracy, precision, detection limit, quantification limit and robustness. Forced degradation of Alpha Lipoic Acid and Enzogenol was carried out under acidic, basic, peroxide, reduction, thermal, photo and hydrolysis conditions. The proposed method is validated as per ICH Q2 (R1) guidelines.
Index Terms- UPLC, Alpha Lipoic Acid and Enzogenol.

INTRODUCTION

Lipoic acid (LA), have some common names like α-lipoic acid, alpha lipoic acid (ALA) and thioctic acid is an organosulfur compound derived from octanoic acid. ALA is essential for aerobic metabolism made in animals. It is manufactured as a dietary supplement, marketed as an antioxidant, and is available as a pharmaceutical drug in other countries [1-7]. It has two sulfur atoms on C6 and C8 carbons and connected by a disulfide bond [8,9] and is thus considered to be oxidized although either sulfur atom can exist in higher oxidation states [10]. The carbon atom at C6 is chiral [11] and the molecule exists as two enantiomers [12] (*R*)-(+)-lipoic acid (RLA) and (*S*)-(-)-lipoic acid (SLA) and as a racemic mixture [13] (*R*/*S*)-lipoic acid (R/S-LA). LA appears physically as a yellow solid and structurally contains a terminal carboxylic acid and a terminal dithiolane ring. For use in dietary supplement materials and compounding pharmacies, the USP [14] has established an official monograph for R/S-LA [15,16].

Fig.1: Structure of Alpha Lipoic Acid

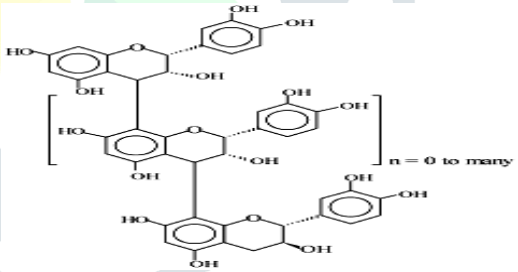

Fig 2: Structure for Enzogenol

Enzogenol is natural complex mixture of plant phenolic compounds contains many different flavonoids [17] and phenolic acids that occur naturally in the pine bark. Phenolic constituents include proanthocyanidins- these are oligo- or polymers of catechins [18]. The proanthocyanidins (often referred to as OPCs = oligomeric proanthocyanidins) are the most abundent group of phenolics in Enzogenol with more than 80% by weight. Further, a diverse range of other flavonoids and related phenolics present in Enzogenol include the monomeric catechins, quercetin [19], dihydroquercetin, myricetin [20], some stilbenes, hydroxylstilbenes, and phenolic acids. By the literature search there was no article published so far for the references. The purposed method was simple and economical sensitive for the estimation of Alpha Lipoic Acid and Empergol.

MATERIALS AND REQUIREMENTS

Instrument:

UPLC, make: Acquity UPLC system consisting of quaternary pump, PDA detector and chromatographic software Empower-2.0 was used.

Reagents:

Acetonitrile (UPLC grade), Water (UPLC grade).

Mobile Phase Preparation: Water: Acetonitrile (40:60).

Mobile Phase-A: Water

Mobile Phase-B: Acetonitrile

Diluent Preparation: Mix Mobile phase-A and Mobile phase-B in 40:60 v/v.

Optimization of mobile phase:

Different trails have done, different buffers and different mobile phases were used to develop the method. In all trails peaks are not separated properly. Finally for the proposed method all the peaks are separated and the entire suitability conditions are within the limit.

Chromatographic conditions:

The chromatographic system was carried out in Waters X-Terra RP18, (150x4.6mm, 2.1µm) column. Flow rate was maintained at 1.0ml/min injection volume is 10µl and sample and column temperatures are ambient. Wavelength detection is maintained at 228 nm.

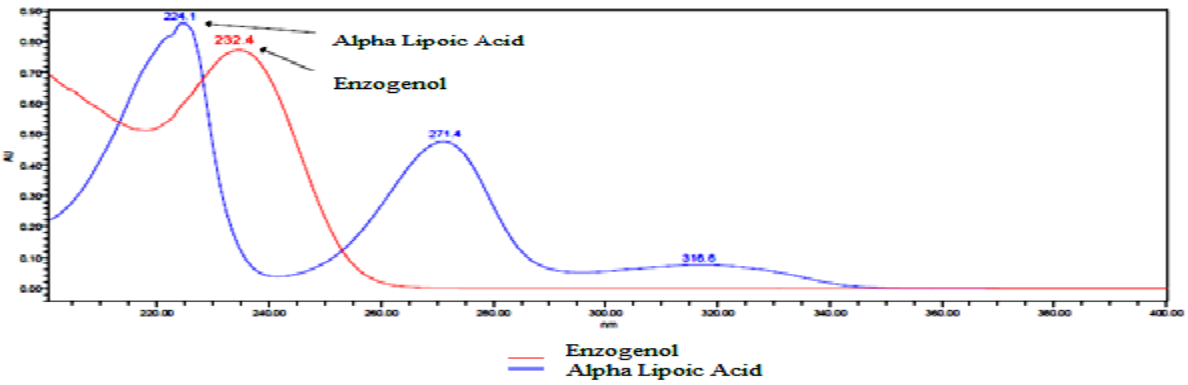

Fig 3: PDA Spectra for Alpha Lipoic Acid and Enzogenol

Standard Solution:

Weigh accurately 150 mg of Alpha Lipoic Acid and 12.5mg of Enzogenol. These working standards were transferred into a 100 ml volumetric flask, add 70 ml of diluent sonicated for 10 min to dissolve the contents make up to the mark with diluent. Further diluted 5ml of above solution to 50ml with diluent.

Sample Solution:

Transfer 175.4 mg of sample into a 100 ml volumetric flask diluted to volume with diluent. Filter through 0.45µ nylon syringe filter.

RESULTS AND DISCUSSION

Validation of proposed method

The method was validated for parameters like System suitability, Specificity, Linearity, LOD, LOQ, Precision, Accuracy, Robustness and Ruggedness as per ICH guidelines [21-22].

System Suitability

The UPLC system was stabilized for 60 min to get a stable baseline. Six replicate injections of standard solution were injected. The results are summarized below table 1.

System Suitability parameter	Acceptance criteria	Drug Name	
		Alpha Lipoic Acid	Enzogenol
% RSD	NMT 2.0	0.209	0.138
USP Tailing	NMT 2.0	1.08	1.09
USP Plate Count	NLT 3000	3126	3958

Table 1: System Suitability Data

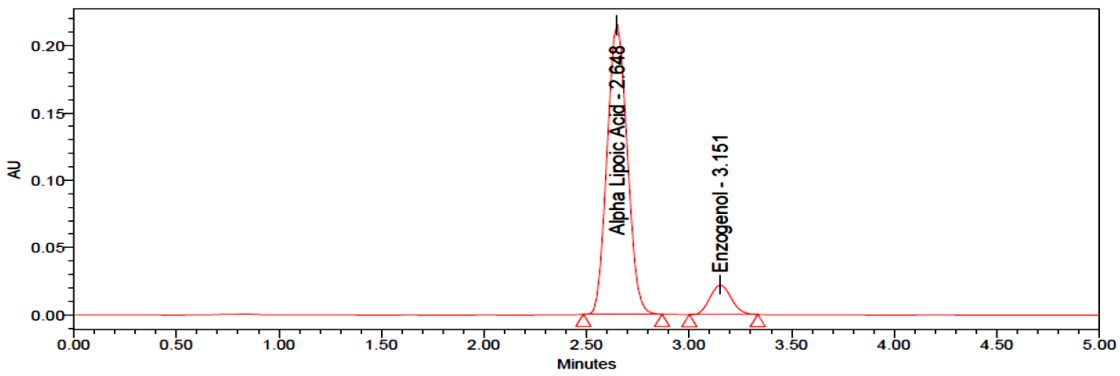

Fig.4: Chromatogram for System suitability

Specificity

There is no interaction of peaks in blank and standard, sample, placebo chromatograms in the total runtime of chromatogram. Hence its proves that method is specific.

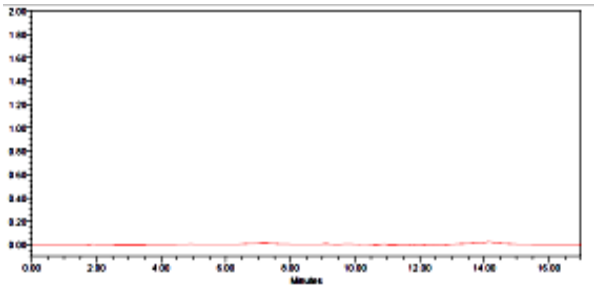

Fig 5: Chromatogram for Blank

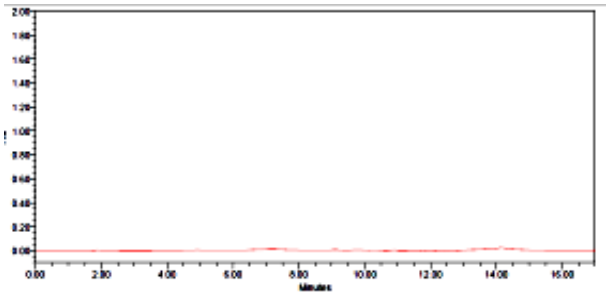

Fig 6: Chromatogram of Placebo

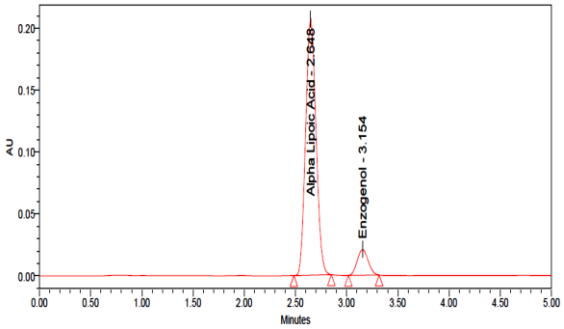

Fig 7: Chromatogram for Standard

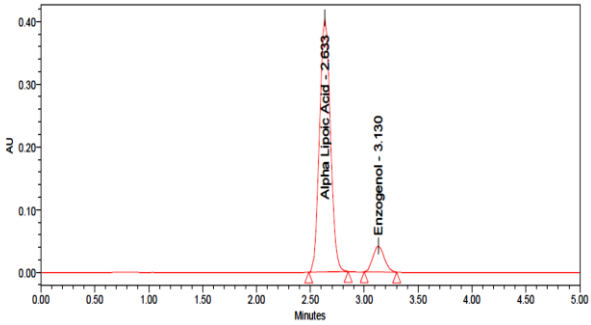

Fig 8: Chromatogram for Sample

Linearity

The linearity was observed in the concentration range of 15µg/ml to 225µg/ml for Alpha Lipoic Acid. The regression equation is Y=18969X+9335 and correlation coefficient was found to be 0.99996.

Enzogenol concentration range from 1.25 µg/ml to 18.75µg/ml, regression equation is Y=24231X+929 and correlation coefficient was found to be 0.99994.

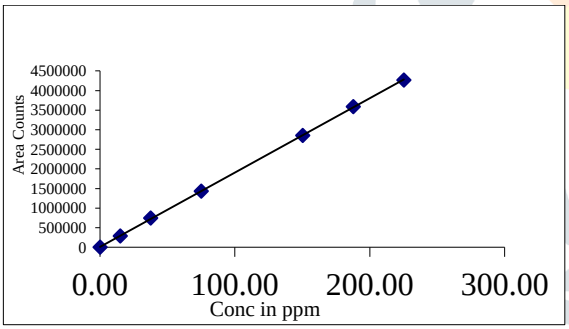

Fig 9: Linearity Plot for Alpha Lipoic Acid

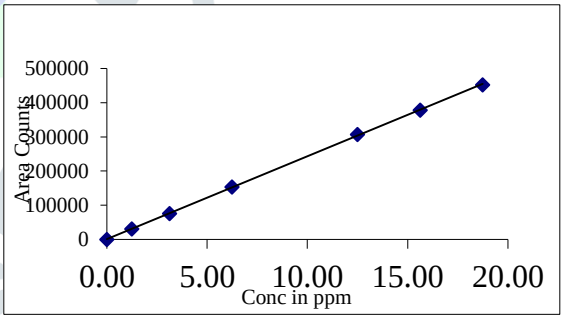

Fig 10: Linearity Plot for Enzogenol

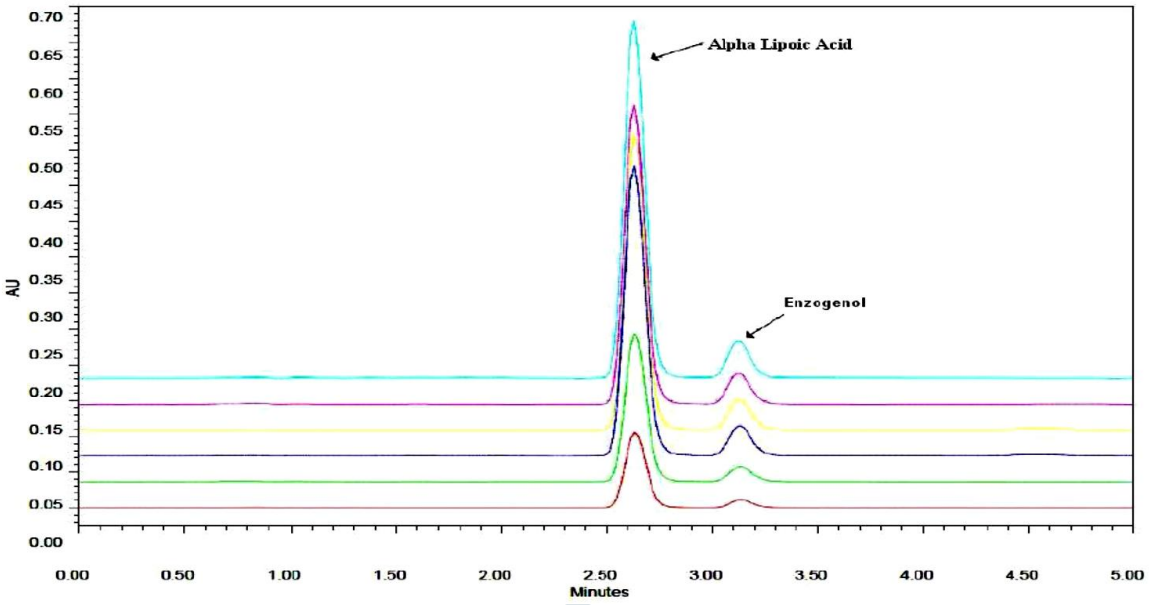

Fig 11: Overlay chromatogram for Linearity

Accuracy

The accuracy of the assay test procedure was determined by Alpha Lipoic Acid and Enzogenol stock solution to test the sample. Injecting samples in triplicate at 50%, 100% and 150% of the target concentration. The recovery results should be NLT 95.0% and NMT 105.0%.

S.No.	% Level	% Recovery	Avg. %Recovery
1	50	100.2	100.4
2		100.6	
3		100.4	
4	100	99.9	100.2
5		100.2	
6		101.6	
7	150	100.5	100.5
8		100.2	
9		100.8	

Table 2: Accuracy results for Alpha Lipoic Acid

S.No.	% Level	% Recovery	Avg. %Recovery
1	50	100.0	100.5
2		100.4	
3		100.0	
4	100	101.0	100.2
5		100.7	
6		100.0	
7	150	100.6	100.0
8		100.0	
9		100.5	

Table 3: Acc results for Enzogenol

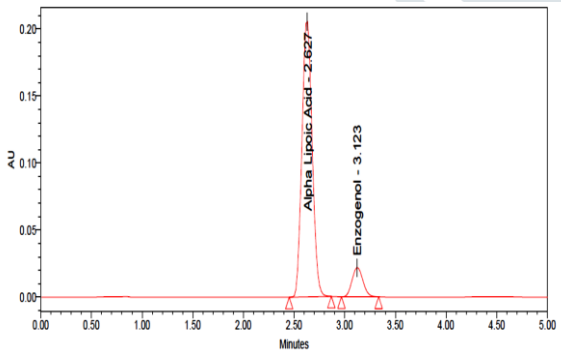

Fig 12: Chromatogram for Accuracy 50%

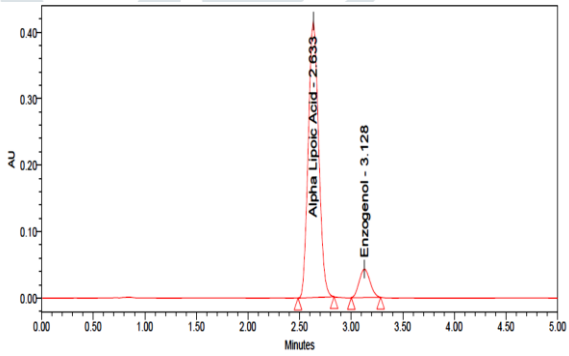

Fig 13: Chromatogram for Accuracy 100%

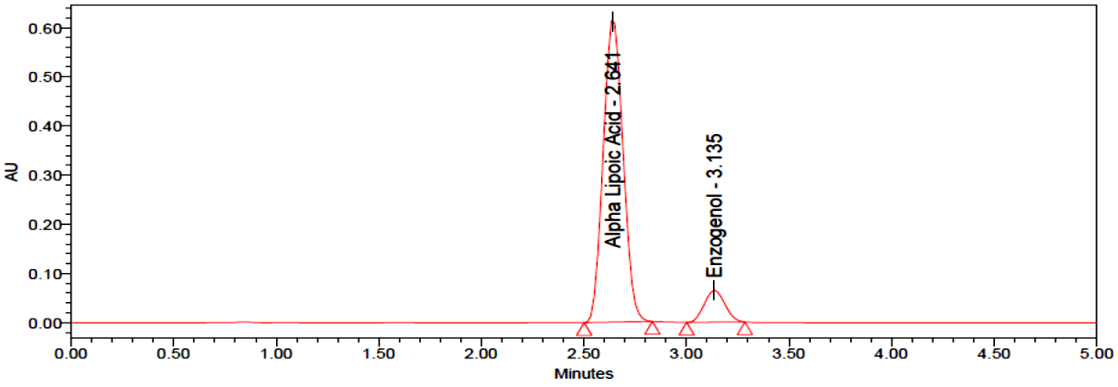

Fig 14: Chromatogram for Accuracy 150%

Precision

Precision of the test method was determined by injecting test preparation and tested through the complete analytical procedure from sample preparation to the final result. Repeatability assessed using a minimum of 6 determinations and calculated % relative standard deviation of assay. Assay results meet the specification limits.

Analyte	Amount Present	% Assay as is (mean)	% RSD of Assay
Alpha Lipoic Acid	150.2	100.7	0.16
Enzogenol	12.5	100.4	0.40

Table 4: Method Precision results

Intermediate Precision

Six replicates of a sample solution were analysed on a different day, different analyst and different instrument. Peak areas were calculated which were used to calculate mean, % RSD values. The results are given below table 5.

Analyte	Amount Present	% Assay as is (mean)	% RSD of Assay
Alpha Lipoic Acid	150.2	100.8	0.5
Enzogenol	12.5	100.2	0.34

Table 5: Intermediate Precision results

LOD and LOQ

LOD and LOQ were separately determined by calibration curve method [23]. LOD and LOQ of the compound were determined by injecting progressively lower concentrations of standard solutions using developed UPLC method. The LOD concentrations for Alpha Lipoic Acid is 0.1502µg/ml the s/n value is 7 and Enzogenol is 0.0125 µg/ml the s/n value is 3. The LOQ concentration for Alpha Lipoic Acid is7.51 µg/ml the s/n values are 28 and Enzogenol is 0.1501 µg/ml the s/n value is 23.

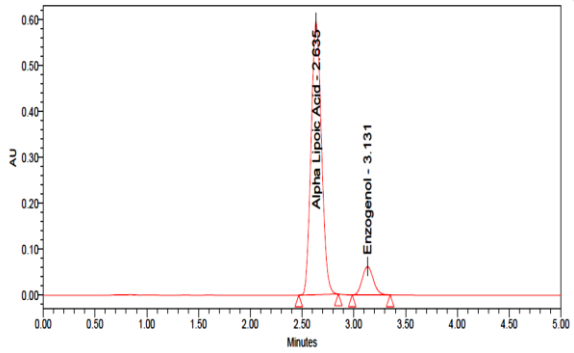

Fig 15: Chromatogram for LOD

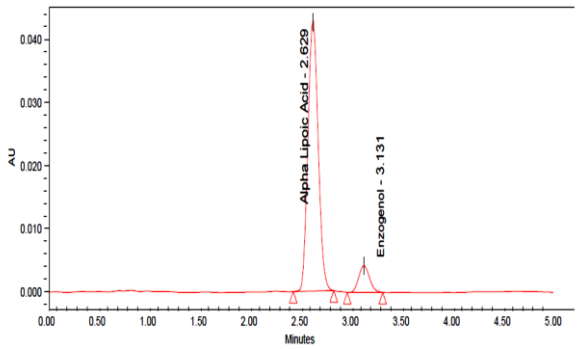

Fig 16: Chromatogram for LOQ

Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the UPLC pump flow rate (±0.2 ml) and organic solvent content (±10%). The alterations caused a significant change in peak area R.S.D (%), USP tailing factor and retention times.

Drug Name	Flow Plus (1.2ml/min) %RSD	Flow Minus (0.8ml/min) %RSD	Organic Plus (45:55) %RSD	Organic Minus (35:65) %RSD	Wave length Plus (233nm) %RSD	Wave lengthMinus (223nm) %RSD
Alpha Lipoic Acid	0.52	0.74	0.51	0.58	0.47	0.54
Enzogenol	1.32	0.94	0.73	1.07	1.54	1.20

Table 6: Robustness data

Stability

The stability of Alpha Lipoic Acid and Enzogenol in solution was determined by sample solution stability initial to 24hr at different time intervals at room temperature. There are no effects in storage conditions for Alpha Lipoic Acid and Enzogenol drugs.

Stability	% of Label claim (Alpha Lipoic Acid)	% of Deviation	% of Label claim (Enzogenol)	% of Deviation
Initial	99.67	0.00	99.55	0.00
6Hr	99.54	0.13	99.51	0.04
12Hr	99.51	0.16	99.43	0.12
18Hr	99.45	0.22	99.40	0.15
24Hr	99.39	0.28	99.35	0.20

Table 7: Results for Solution Stability

Forced Degradation

The Alpha Lipoic Acid and Enzogenol sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Forced degradation studies were performed to show the method is suitable for degraded products. Moreover, the studies provide information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities [24].

Acid Degradation:

10 ml of sample transferred into a 100 ml volumetric flask add 10ml of 0.1N HCl heat for 15min at 60ºC after that add 10 ml of 0.1N NaOH then makeup to mark with diluent. Then the solution is filter through 0.45µ nylon syringe filter.

Alkali Degradation:

10ml of sample transferred into a 100 ml volumetric flask add 10 ml of 0.1N NaOH heat for 15min at 60ºC after that add 10ml of 0.1N HCl then makeup to the mark with diluent. Then the solution is filter through 0.45µ nylon syringe filter.

Peroxide Degradation:

10ml of sample transferred into a 100ml volumetric flask add 5ml of 10%H_2O_2 heat for 30min at 60ºC then cool to makeup with diluent. Filter the solution with 0.45µ nylon syringe filter.

Reduction Degradation:

10ml of sample transferred into a 100ml volumetric flask add 10ml of 10% sodium bicarbonate solution heat for 15min at 60ºC then cool to makeup with diluent. Filter the solution with 0.45µ nylon syringe filter.

Thermal Degradation:

The sample drug solution was placed in oven at 105ºC for 6Hr. The resultant solution was injected into UPLC system.

Photolytic Degradation:

The sample solution was exposed into sunlight for 6hr. The sample was injected into UPLC system.

Degradation	Alpha Lipoic Acid (% Assay)	% Degradation	Enzogenol (% Assay)	% Degradation
Control	101.1	0.00	100.4	0.00
Acid	88.4	12.7	81.4	19.0

Alkali	83.6	17.5	82	18.4
Peroxide	88.7	12.4	85.7	14.7
Reduction	83	18.1	85	15.4
Thermal	84.1	17.0	82.4	18.0
Photo	83.8	17.3	89.6	10.8
Humidity	83.4	17.7	81	19.4
Hydrolysis	85.3	15.8	83.5	16.9

Table 8: Results for Forced Degradation

Conclusion

The developed method gave good resolution between Alpha Lipoic Acid and Enzogenol with short runtime (5min), high efficiency and complies with modified SST specifications of USP. The use of RP18 column in the present work has shown better elution of analytes with more resolution, improved plate count, tailing. So the RP18 column can be used to achieve high specificity in shorter time of analysis of Alpha Lipoic Acid and Enzogenol as per ICH Q3A (R2) [25] guidelines. The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination and quantification of Alpha Lipoic Acid and Enzogenol. The sample recovery was in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Alpha Lipoic Acid and Enzogenol in combined dosage form.

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