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STABILITY INDICATING HPLC DEPENDENT METHOD FOR QUALITY CONTROL QUANTIFICATION OF ETHINYLOESTRADIOL AND DIGENOEST IN COMBINATION FORMULATIONS

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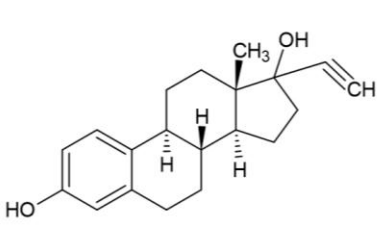
ABSTRACT

Objective: An HPLC DNGT/EETL assay technique has been established for the assessment of DNGT and EETL in combination formulations. The Cosmicsin column (150 mm × 4.5 mm; 4.5 µ size) operating at room temperature and acetonitrile/phosphate buffer (0.1 M, pH 4.2, KH_2PO_4) with ratio of 45/55 *v/v* as mobile phase was used for DNGT and EETL separation and analysis. The flow rate about 1.0ml/min and the detection wavelength at 242 nm was employed. The EETL and DNGT were eluted at 1.760 and 2.309 min, respectively. With an R2 > 0.9999, a straight line relationship was found for the concentration ranges of 15 to 45 µg/ml (EETL) and 100 to 300 µg/ml (DNGT). The sensitivity could be seen using LOQ, which had values of 0.277 µg/ml (EETL) and 0.344 µg/ml (DNGT). The RSD <2% and recovery rates ranging from 99.77% to 101.10, respectively, demonstrated the accuracy and precision The stabilities of DNGT and EETL were tested under scenarios of basic, acidic, oxidative, photolytic and thermal stress. The procedure for DNGT and EETL analysis were verified in compliance with Q2R1 ICH guidelines. The suggested HPLC DNGT/EETL test procedure proved appropriate for the assessment of DNGT and EETL simultaneously during quality control and stability investigations.

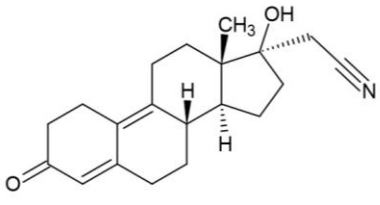
KEYWORDS: Acne, Ethinylestradiol, Dienogest, Stability indicating, Quality control.

INTRODUCTION

The most commonly encountered chronic inflammatory dermatological disorder which impacts teenagers and young adults is acne vulgaris. Reports on the general incidence of this illness vary from 12% to 59%.[1-3] The "European Medicines Agency" recommended on January 26, 2016, that medicinal products encompassing a combined dose of 0.03 mg ethinylestradiol (EETL, Fig. 1) and 2 mg dienogest (DNGT, Fig. 1) could potentially be employed for mild to moderate acne breakouts in cases when topical therapies nor oral antibiotics have failed.[4,5]



Ethinylestradiol
IUPAC name:
(8R,9S,13S,14S,17R)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-4,17-diol



Dienogest
IUPAC name:
2-[(8S,13S,14S,17R)-17-hydroxy-13-methyl-3-oxo-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta[a]phenanthren-17-yl]acetonitrile

Fig. 1: IUPAC names and structures of EETL and DNGT.

Quality control testing are vital to make sure that tablets manufactured fulfil requirements for patient acceptability, safety, effectiveness, and uniformity in drug content across tablets, as well as that the drugs will release and also dissolve as intended, quality control testing are essential.[6,7] LC-MS method by Laszlo et al.[8], UPLC-MS/MS method by Nair et al.[9], RP-HPLC methods by Laban et al.[10], Chandran et al.[11], Suchitra & Satyanarayana[12], spectrophotometric methods by Saranya et al.[13] and Cağlayan et al.[14] offered an assessment of EETL and DNGT either separately or in conjunction with additional contraceptive agents. Laszlo et al.[8] and Nair et al.[9] methods are applicable to murine or human plasma. Laban et al.[10] Cağlayan et al. Chandran et al.[11], Suchitra & Satyanarayana[12], Saranya et al.[13] and Cağlayan et al.[14] were applicable to contraceptive formulations.

Quantifying DNGT and EETL in combination formulations was not done using any of the approaches.[8-14] Considering the benefits of HPLC, there was a significant demand for a sensitive, selective, and stability-indicating approach with a broad linear dynamic scope of application. The HPLC DNGT/EETL assay technique capacity for demonstrating stability-indicating was tested under scenarios of basic, acidic, oxidative, photolytic and thermal stress.

MATERIALS AND METHODS

Equipment and chemicals

The method development and validation process for DNGT and EETL analysis was carried out using the HPLC model [Waters, USA]. Empower software 2.0 was used to procure the data from the DNGT and EETL chromatograms. In the present investigation, C_2H_3N (acetonitrile), CH_3OH (methanol), HCl (hydrochloric acid), KH_2PO_4 (potassium dihydrogen phosphate), NaOH (sodium hydroxide), and H_2O_2 (peroxide) were employed. All of the chemicals employed, which were of HPLC and analytical quality, were bought from Merck Ltd. in India.

Reference drug molecules and its formulations

DNGT – 2.0mg and EETL – 0.03mg, as stated on the label of Alembic Pharmaceuticals Ltd.'s commercial product "Estroplus", were used. The reference DNGT and EETL molecules were offered from "Hetero drug pvt ltd" (India).

Conditions for DNGT and EETL analysis

The Cosmicsin column (150 mm × 4.5 mm; 4.5µ size) operating at room temperature, the acetonitrile/phosphate buffer (0.1 M, pH 4.2, KH_2PO_4) ratio of 45/55 %*v/v* as mobile phase, flow rate about 1.0ml/min, the detection wavelength at 242 nm, and the sample injection rate of 10 µL were employed for DNGT and EETL analysis. An 0.22-micron pore-size nylon membrane filter was expended to filter the mobile phase and then degassed.

Stock and working DNGT and EETL solutions

The stock DNGT (2000 µg/ml) and EETL (300 µg/ml) solution was made by weighing the APIs (DNGT – 200 mg and EETL – 30 mg) and blending them with the mobile phase (100 ml). Stock DNGT (2000 µg/ml) and EETL (300 µg/ml) solution were diluted appropriately with diluent to create working DNGT (200 µg/ml) and EETL (30 µg/ml) solution.

Calibration curve

The stock DNGT (2000µg/ml) and EETL (300µg/ml) solution was put to use for generating a range of concentrations for DNGT (100 to 300µg/ml) and EETL (15 to 45 µg/ml). The concentrations generated were: for DNGT – 100 µg/ml; 150 µg/ml; 200 µg/ml; 250µg/ml; 300 µg/ml and for DNGT – 15µg/ml; 22.5µg/ml; 30 µg/ml; 37.5µg/ml; 45 µg/ml. The calibration curves for DNGT and EETL were created by plotting the concentration to area. Equations for regression were developed for the DNGT and EETL by utilizing the acquired calibration data.

Analysis of DNGT and EETL in Estroplus tablets

Ten Estroplus tablets were finely powdered. An amount of Estroplus powder equivalent to 200mg DNGT and 30mg EETL were perfectly weighed. Transferred that Estroplus powder to 100 ml capacity flask and then added 50 ml of mobile phase. The solution had been sonicated over 15 min before being diluted to volume utilizing distilled water. An 0.22-micron pore-size nylon membrane filter was expended to filter the Estroplus tablet solution. Theoretical concentrations (DNGT - 200µg/ml and EETL - 30µg/ml) was obtained for analysis by diluting stock Estroplus tablet (DNGT - 2000 µg/ml and EETL -300 µg/ml) solution with the mobile phase. The chromatographic methodology in section "Conditions for DNGT and EETL analysis" was implemented to analyze the DNGT and EETL contents of Estroplus tablets.

Forced degradation/Selectivity studies

To assess the specificity and even stability-indicating properties of the DNGT and EETL assay HPLC methodology, forced degradation analyses under a range of stress conditions were carried out, including basic (using 0.1N NaOH) stress, acidic (0.1 N HCl) stress, neutral (using distilled water) stress, oxidative (using 30% H_2O_2) stress, thermal (at temperature 80 ^oC) stress, and photolytic (exposure to sun light) stress conditions. As advised by the ICH, the degradation investigations on DNGT and EETL were performed under mild conditions.[15]

Preparation of drug for Acid stress, Alkali stress, Neutral stress, Oxidative stress

The DNGT and EETL was degraded with acid by blending 10 ml of stock Estroplus tablet (DNGT - 2000µg/ml and EETL - 300µg/ml) with 10 ml of 0.1N HCl at 60 ^oC temperature for almost 30 min.

Thermal and photo stress

An amount of Estroplus powder equivalent to 200mg DNGT and 30mg EETL were perfectly weighed, exposed to 80 ^{o}C in oven for 6 hr to apply thermal stress on DNGT and EETL or to direct sun light for 6 hr to apply photo stress on DNGT and EETL. Transferred that degraded Estroplus powder to 100 ml capacity flask and then added 50 ml of mobile phase. The solution had been sonicated over 15 min before being diluted to volume utilizing again the mobile phase. An 0.22-micron pore-size nylon membrane filter was expended to filter the degraded Estroplus tablet solutions. Theoretical concentrations (DNGT - 200µg/ml and EETL - 30µg/ml) was obtained for analysis by diluting degraded Estroplus tablet (DNGT - 2000µg/ml and EETL - 300µg/ml) solution with the mobile phase.

The chromatographic methodology in section "Conditions for DNGT and EETL analysis**"** was implemented to analyze the contents of DNGT and EETL in all degraded Estroplus tablet solutions.

RESULTS

"Conditions for DNGT and EETL analysis" – Development

At 242 nm, DNGT and EETL showed significant absorption. The DNGT and EETL analyses were done at exactly the same nanometers (242 nm). The temperatures (sample and column – 25^{o}C), flow rate about 1 ml/min and the sample injection rate of 10 µL was kept constant throughout all of the experimental trail runs.

When "Waters" column (C18; 250mm × 4.5mm; 4.5 µ size) and methanol/phosphoric acid (0.01%) ratio of 40/60 %*v/v* as mobile phase was employed, one peak was eluted. This time "Inertsil" column (C18; 250 mm × 4.5 mm; 4.5 µ size) was used. All other conditions stayed the same. DNGT and EETL peaks appeared, however their shapes weren't suitable. A "YMC" column (C18; 150 mm × 4.5 mm; 4.5 µ size) with an acetonitrile/NaH_2PO_4 (0.1M) in ratio of 40/60 *v/v* used as the mobile phase produced DNGT and EETL peaks, however the DNGT peak wasn't appropriate. When "Cosmicsin" column (C18; 150 mm × 4.5 mm; 4.5 µ size) and acetonitrile/ KH_2PO_4 (0.1M) ratio of 30/70 *v/v* as mobile phase was employed, DNGT and EETL peaks appeared, however DNGT peak shows more tailing and also observed disturbance in base line. When "Cosmicsin" column (C18; 150 mm × 4.5 mm; 4.5 µ size) and acetonitrile/ KH_2PO_4 (0.1M, pH 4.2) ratio of 45/55 *v/v* as mobile phase was employed, DNGT and EETL peaks appeared good (Fig. 2). Acceptable results were seen for the resolution, tailing, and plate count. For DNGT and EETL analysis, these specifications are selected as a result.

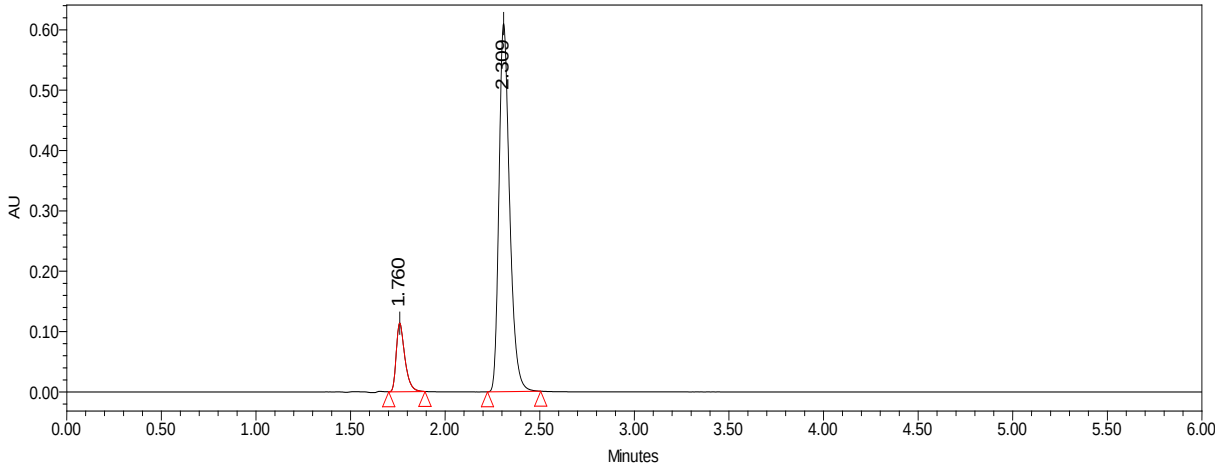


Fig. 2: Chromatogram with EETL and DNGT eluted at 1.760 and 2.309 min.

"Conditions for DNGT and EETL analysis" – Validation

The procedure ("Conditions for DNGT and EETL analysis") were verified in compliance with Q2R1 ICH guidelines.[16]

Selectivity

The blank (acetonitrile 45% vol and 0.1M KH_2PO_4 55% vol), working DNGT (200 µg/ml) and EETL (30µg/ml) solution and Estroplus tablet (DNGT - 200µg/ml and EETL - 30µg/ml) solution were injected, analysed applying optimized "Conditions for DNGT and EETL analysis" and respective chromatograms were collected (Fig.3). Peak retention durations for DNGT and EETL in the working and Estroplus samples are almost equal, and no peak existed in the blank.

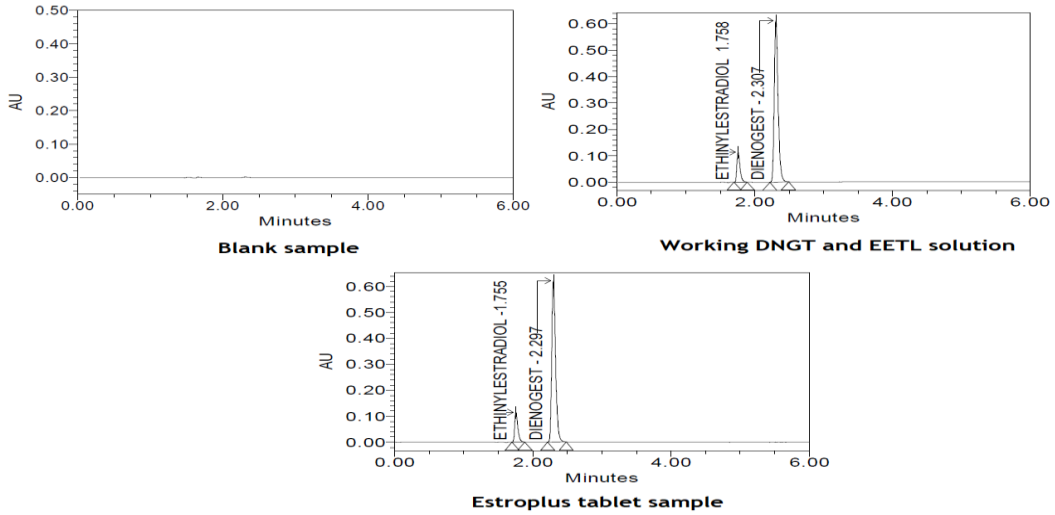


Fig. 3: Chromatograms of blank; working DNGT and EETL solution and Estroplus tablet solution

Linearity

At five diverse levels (DNGT - 100 to 300 µg/ml and EETL - 15 to 45 µg/ml) of concentration, calibration DNGT and EETL solutions were made. The peak areas against concentrations information of DNGT and EETL were evaluated by means of linear regression investigation (Table 1). Fig.4 illustrates the DNGT and EETL linearity plots.

Table 1: Linearity and regression parameters of DNGT and EETL.

EETL concentration (µg/ml)	EETL peak area	DNGT concentration (µg/ml)	DNGT peak area
15	177745	100	1169837
22.5	266245	150	1762575
30	356910	200	2354416
37.5	445109	250	2957378
45	535146	300	3543785
Regression parameters: **Slope:** 11915.546 **Intercept:** - 1235.4		**Regression parameters:** **Slope:** 11885.398 **Intercept:** - 19481.4	

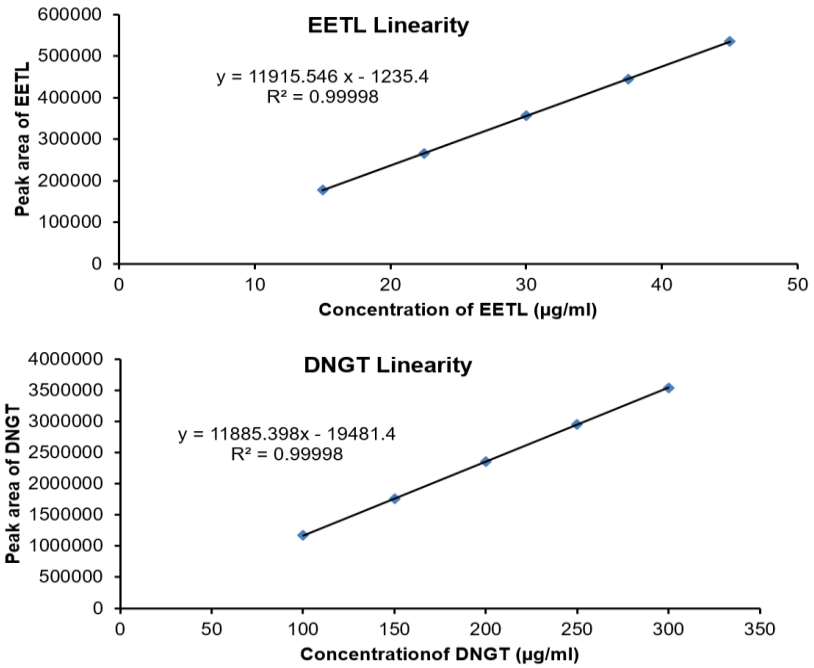


Fig. 4: EETL and DNGT linearity plots.

Sensitivity

The ratio between signal:noise served for calculating LOD & LOQ. The DNGT and EETL concentration is considered LOD, if its ratio between signal:noise is ≥3, and LOQ if it is ≥10. The LOD measured were 0.103µg/ml (3.8 signal:noise) 0.083 µg/ml (3.2 signal:noise) for DNGT and EETL, respectively. The LOQ measured were 0.344 µg/ml (10.8 signal:noise) 0.277µg/ml (10.4 signal:noise) for DNGT and EETL, respectively. Fig.5 illustrates the respectively DNGT and EETL chromatograms.

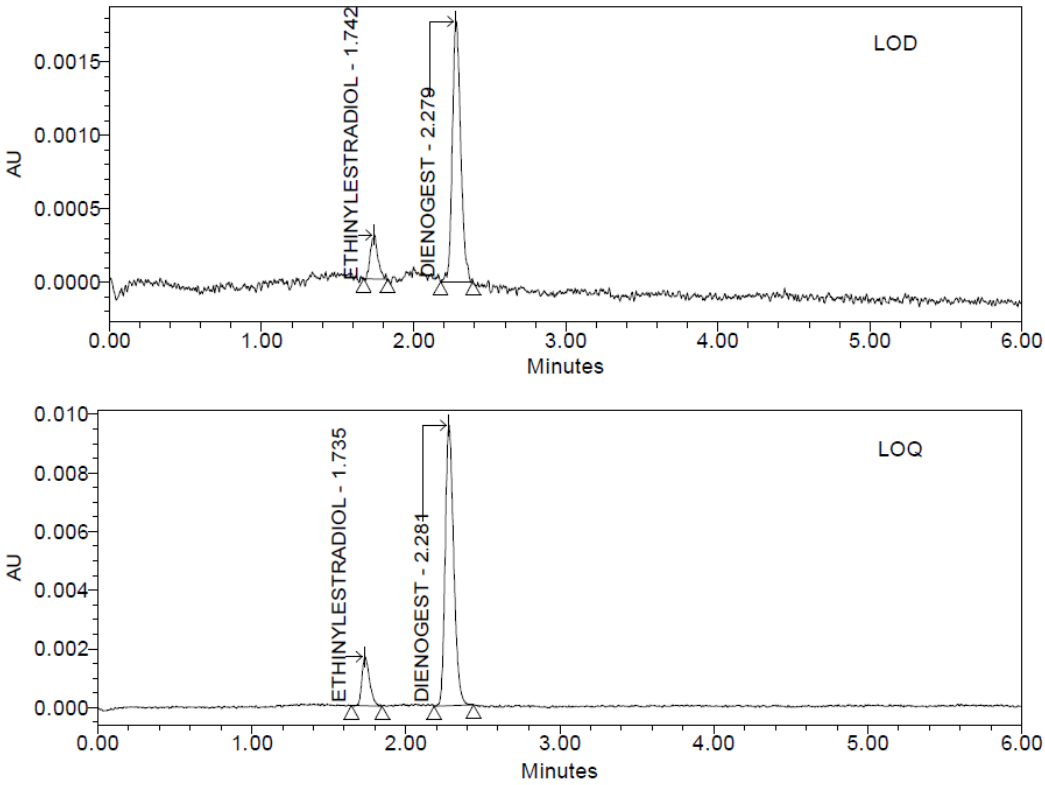


Fig. 5: DNGT and EETL chromatograms at concentrations LOD and LOQ

Precision

The process of DNGT and EETL analysis by applying optimized "Conditions for DNGT and EETL analysis" was repeated six times using a working DNGT (200 µg/ml) and EETL (30 µg/ml) solution on the same day for the purpose to assess the precision. The results (mean peak; SD; and RSD) of precision experiments for DNGT and EETL are summarized in Table 2.

Table 2: Precision and accuracy experiment results for DNGT and EETL.

Sample injection.	DNGT analysis		EETL analysis	
	Precision	Accuracy	Precision	Accuracy
	Peak area	Assay (%) found	Peak area	Assay (%) found
1	2358429	99.201	356432	98.743
2	2346892	99.318	356852	98.260
3	2353600	98.975	355621	98.541
4	2341791	98.915	355405	98.047
5	2354768	99.125	356158	98.590
6	2350723	99.048	355881	98.421
Average	2351034	99.097	356058	98.434
STD.Dev	5964.882	0.149	534.606	0.250
RSD (%)	0.2537	0.150	0.150	0.254

Accuracy

A working DNGT (200µg/ml) and EETL (30µg/ml) solution sample was repeatedly injected (six times), analysed by applying optimized "Conditions for DNGT and EETL analysis" and determined the %assay for the DNGT and EETL peak area measurements obtained. This process was used to assess the accuracy. The results (mean assay%; SD; and RSD) of accuracy experiments for DNGT and EETL are summarized in Table 2.

Recovery study

The known quantities of DNGT (98.0µg/ml quantity, 196.0µg/ml quantity and 294.0 ppm µg/ml) and EETL (14.85µg/ml quantity, 29.70µg/ml quantity and 44.55µg/ml quantity), were added to a Estroplus tablet solution, ranging from 50% to 150%. The results (mean %recoveries) of recovery experiments for DNGT and EETL are summarized in Table 3.

Table 3: DNGT and EETL recovery outcomes.

Drug	Added drug quantity (µg/ml)	Found drug quantity (µg/ml)	Recovered drug (%)	Average (%)
50% level assessments				
DNGT	98.00	97.67	99.66	99.98
	98.00	97.79	99.79	
	98.00	98.47	100.48	
EETL	14.85	14.81	99.73	99.77
	14.85	14.77	99.45	
	14.85	14.87	100.13	
100% level assessments				
DNGT	196.00	196.83	100.42	100.35
	196.00	196.87	100.44	
	196.00	196.35	100.18	
EETL	29.70	29.76	100.21	100.00
	29.70	29.68	99.94	
	29.70	29.66	99.86	
150% level assessments				
DNGT	294.000	296.79	100.95	101.10
	294.000	296.95	101.00	
	294.000	297.99	101.36	
EETL	44.55	44.72	100.38	100.31
	44.55	44.75	100.44	
	44.55	44.60	100.12	

Robustness

To test the robustness of analysis towards different parameters, the samples, working DNGT (200 µg/ml) and EETL (30 µg/ml) solution sample, were analysed using three different:

- Column temperatures - 23 ^{o}C; 25 ^{o}C; and 47 ^{o}C
- Flow rates – 0.9; 1.0; and 1.1 ml/min
- Acetonitrile volumes – 40% ratio; 45% ratio; and 50% ratio
- Wavelengths – 240 nm; 242 nm; and 244 nm
- pH – 4.0; 4.2; and 4.4

The results (mean peak; mean tailing; mean plate counts; SD; and RSD) of robustness experiments for DNGT and EETL are summarized in Table 4.

Table 4: Robustness outcomes for DNGT and EETL.

Drug with quantity↓	Parameter modified	Statistical parameter	Values obtained for		
			Tailing	Area	Plate count number
DNGT (200 µg/ml)	Temperature	Mean (n=3 findings)	1.3	2384101	3187.2
		±SD/RSD%	±0.01/0.8%	±45695.6/1.9%	±118.7/1.5%
	Wavelength	Mean (n=3 findings)	1.3	2371436.7	8285.3
		±SD/RSD%	±0.1/0.4%	±21972.7/0.9%	±34.2/0.4%
	Flow rate	Mean (n=3 findings)	1.3	2381866.0	8149.4
		±SD/RSD%	±0.03/1.5%	±45062.6/1.9%	±94.4/1.2%
	pH	Mean (n=3 findings)	1.3	2380194.9	8376.7
		±SD/RSD%	±0.01/0.4%	±4456.0/0.2%	±132.5/1.6%
	Methanol ratio	Mean (n=3 findings)	1.3	2392744.3	8232.7
		±SD/RSD%	±0.02/1.0%	±39188.3/1.6%	±112.8/1.4%
EETL (30 µg/ml)	Temperature	Mean (n=3 findings)	1.7	358607.7	7366.7
		±SD/RSD%	±0.01/0.7%	±5928.1/1.7%	±56.4/0.8%
	Wavelength	Mean (n=3 findings)	1.5	359044.3	7353.3
		±SD/RSD%	±0.02/0.4%	±6201.1/1.7%	±30.2/0.4%
	Flow rate	Mean (n=3 findings)	1.5	358503.7	7269.7

		±SD/RSD%	±0.02/1.0%	±6578.3/1.8%	±123.6/1.7%
	pH	Mean (n=3 findings)	1.5	358893.9	7381.6
		±SD/RSD%	±0.01/0.4%	±484.4/0.1%	±108.7/1.5%
	Methanol ratio	Mean (n=3 findings)	1.5	358437.0	7266.7
		±SD/RSD%	±0.01/0.7%	±5693.7/1.6%	±120.4/1.7%

System suitability

The same working DNGT (200 µg/ml) and EETL (30 µg/ml) solution were injected, analysed by applying optimized "Conditions for DNGT and EETL analysis" in five replicates. Following this, the system appropriateness characteristics (%RSD, theoretical plates, retention time, resolution and tailing factor) were computed for both DNGT and EETL. The results (mean peak; mean tailing; mean plate counts; mean resolution; SD; and RSD) of suitability experiments for DNGT and EETL are summarized in Table 5.

Table 5: Evaluation of optimal conditions for system appropriateness.

Drug→	**DNGT**		**EETL**		**Limits**
Parameter↓	**Mean ± SD***	**RSD**	**Mean ± SD***	**RSD**	
Resolution (R)	5.846±0.0152	0.2592	-	-	R ≥ 2
Theoretical plates (N)	8440±103.3383	1.2237	7445±41.5054	0.5575	N ≥ 2000
Tailing (Tf)	1.314±0.0089	0.6807	1.474±0.0055	0.3716	0.8 < Tf < 2
Retention time (min)	2.307±0.0021	0.0899	1.759±0.0015	0.0862	%RSD ≤ 2
Area	2378890±2512.2551	0.1056	358225±825.0507	0.2303	%RSD ≤ 2

*Mean and SD of five repetitions using the same working DNGT and EETL standard.

Stability of DNGT and EETL in forced degradation

Forced degradation analyses under a range of stress conditions were carried out, including basic (using 0.1N NaOH) stress, acidic (using 0.1 N HCl) stress, neutral (using distilled water) stress, oxidative (using 30% H_2O_2) stress, thermal (at temperature 80 ^oC) stress, and photolytic (exposure to sun light) stress conditions on Estroplus tablet solution.

Overall, during photostress degradations, 95.46% of EETL was retained while just 4.54% was degraded. It was, therefore. established that EETL was substantially stable under photostress. An 5.62% of EETL was degraded, however 94.38% of it was still present while oxidative stress degradations. Finding that EETL was sufficiently stable despite oxidative stress was the outcome. Alkali-stress degradations appear to have damaged just 7.04% of EETL, leaving 92.96% of the original EETL material intact. EETL was stated to be enough stable under alkali-stress as a consequence. Of the total EETL, 89.17% was not degraded by dry heat, whereas 10.03% had significantly deteriorated. Therefore, it turned out that EETL was unstable when applied dry heat. Of the total EETL, 12.95% was degraded and 87.05% remained following acidic stress degradation. Thus, under acidic degradation, EETL was discovered to be extremely unstable. Degradation of EETL was observed to be minimal (2.44%) under water-induced stress. The EETL is hence more stable (97.56%) despite water-induced stress.

Dry heat conditions caused the DNGT to degrade, with 12.09% and 87.19% of the DNGT persisting in solutions. Thus, under dry heat conditions, DGNT was discovered to be extremely unstable. DNGT degradation was also seen during acidic stress degradations, with 10.02% of the DNGT damaged and 89.98% of the DNGT remaining following deterioration. Therefore, it turned out that DNGT was unstable when applied acidic stress. Oxidative-stress degradations appear to have degraded just 6.80% of DNGT, leaving 93.20% of the original DNGT material intact. DNGT was stated to be enough stable under oxidative-stress. When the Estroplus tablet solution went through exposure to a mild alkali condition, the DNGT hydrolyzed and was lost in a total of 5.39%. After degradation, 94.61% of the DNGT was still present. Enough stability was established for DNGT under alkaline conditions. The percentage of DNGT degraded and remained were 3.28% and 96.72%, respectively, in applied photostress condition. As therefore, it turned out that DNGT was stable when applied sun light. Water-induced stress resulted in very little (1.05%) DNGT degradation. As a result, under stress caused by water, DNGT is more stable (98.95%).

Stability indicating feature

Forced degradation needs to be carried out for the analytes (EETL and DNGT) under various circumstances [basic (using 0.1N NaOH) stress, acidic (0.1 N HCl) stress, oxidative (using 30% H_2O_2) stress, thermal (at temperature 80 ^oC) stress, and photolytic (exposure to sun light)] in order to specify the stability-indicating capabilities of HPLC DNGT/EETL assay technique. The chromatograms acquired under various circumstances are analysed (Fig. 6). When EETL and DNGT were present together with their degradation products, the HPLC DNGT/EETL test method could identify and measure both of them efficiently.

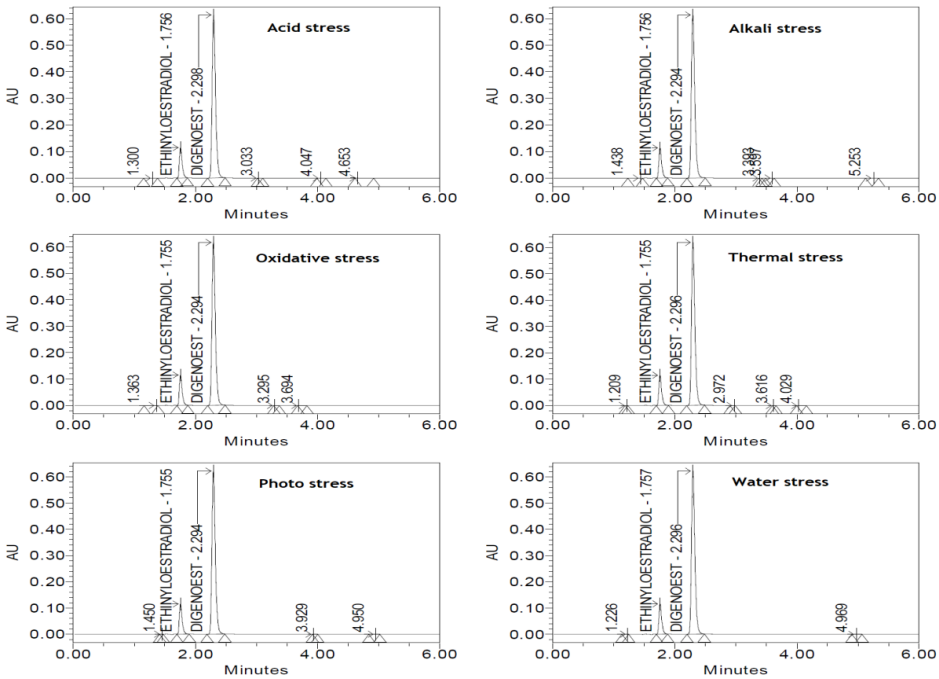


Fig. 6: Chromatograms displaying the EETL and DNGT stress degradation observations.

Peak thresholds, as determined by PDA detector, were from 0.684 to 0.883 for EETL and 0.557 to 0.958 for DNGT across the various degradation scenarios, while peak purity angles, as determined by PDA detector, were in between the values of 0.164 to 0.374 for EETL and 0.151 to 0.381 for DNGT among all degradation scenarios.

DISCUSSION

At the DNGT and EETL peak's retention period (2.307 min of time for DNGT; 1.758 min of time for EETL), neither the blank solvents and excipients of Estroplus tablet showed any interference with the investigated DNGT and EETL peaks. Thus proved specificity for HPLC DNGT/EETL assay technique. The HPLC DNGT/EETL assay technique clearly revealed linearity, with correlation coefficients of 0.9999 for both DNGT and EETL.

Determined outcomes of LOD and LOQ for DNGT and EETL peaks exhibited sensitivity of HPLC DNGT/EETL assay technique towards analysis of investigated molecules (DNGT and EETL). Relative standard deviations, indicative of the precision findings, were consistently smaller than 2.0% for HPLC DNGT/EETL assay technique. By getting percentage recoveries between 99.98% and 101.10% for DNGT, and 99.97% and 100.31% for EETL, the HPLC DNGT/EETL assay technique appeared to be accurate. The percent relative difference that was computed robustness experiments for DNGT and EETL was less than 2.0%, indicating HPLC DNGT/EETL assay technique's robustness towards studied parameters.

An essential component of analytical techniques is the system appropriateness test. The purpose of the evaluation was to indorse that the HPLC DNGT/EETL assay technique is suitable for the DNGT and EETL analysis. The results (mean peak; mean tailing; mean plate counts; mean resolution; SD; and RSD) of suitability experiments for DNGT and EETL are within acceptance gauges.[17]

Following the observation of the Estroplus tablet solution degradation study findings, the order of EETL stability was: neutral stress > photostress > oxidative stress > alkali stress > thermal stress > acid stress. The order of DNGT stability was: neutral stress > photostress > alkali stress > oxidative stress > acid stress > thermal stress. The PDA detector measurements revealed that peak thresholds exceeded compared with peak purity angles of DNGT and EETL in all scenarios of degradation. This indicating HPLC DNGT/EETL assay technique's stability-indicating and specificity towards studied drug analysis.

CONCLUSION

An internal standard-free HPLC DNGT/EETL assay method has been developed for DNGT and EETL evaluation in combination formulations. The DNGT and EETL were evaluated applying the HPLC DNGT/EETL assay approach with excellent recovery, sensitivity, precision, suitability, and selectivity. The established procedure is repeatable, and this feature substantially satisfies the formal standards set out by the ICH. Furthermore, the suggested method's recovery towards DNGT and EETL contents is both feasible as well as achievable. When neutral stress was applied, DNGT and EETL remained stable. In applied acid stress, EETL was the least stable, but in thermal stress, DNGT was the least stable. The stability indicating property of the HPLC DNGT/EETL test technique was shown by the

well-resolved degradants generated under all imposed stress conditions from DNGT and EETL peaks. During quality control as well as stability investigations, the proposed HPLC DNGT/EETL test technique proven suitable for the quantitative measurement of DNGT and EETL in combined formulation, providing quick and precise analysis.

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CONFLICTS OF INTEREST
Nil.

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