ORIGINAL ARTICLE

Development and validation of a simple and precise analytical technique for the simultaneous quantification of drospirenone and estetrol in bulk and tablets using RP-HPLC

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Abstract

Aim: For the simultaneous estimate of drospirenone (DSP) and estetrol (ESR), a new oral contraceptive in bulk and dose forms, a simple methodology was established using RP-HPLC. **Methods:** Analytes were separated using Methanol: Phosphate Buffer pH 6.8 adjusted with dil. NaOH (40:60, v/v) as mobile phase pumped at 1.0 ml/min on a Waters C18 Column with 250 mm 4.6 mm i.d. and 5 m particle size. The column temperature was maintained at 30°C, and a photo diode array detector was used to find an isosbestic point of 215 nm for detection. With a total run time of 6 min, mobile phase was employed as a diluent. Precision, accuracy, linearity, specificity, and robustness of the devised methodology were all validated according to ICH recommendations. To establish the method's stability indicating nature, forced degradation studies were conducted. **Results:** ESR and DSP had retention times of 2.391 and 4.602 min, respectively. Both the drugs exhibited excellent linearity in between 40–120 µg/mL and 189–567 µg/mL for DSP and ESR, respectively. The method was found to be very sensitive. **Conclusion:** As a result, the suggested RP-HPLC method for the quantification of DSP and ESR was reliable, repeatable, accurate, and sensitive.

Key words: Drospirenone, Estetrol, Oral contraceptives, Methanol, Phosphate buffer

INTRODUCTION

The primary form of contraception involves combining the effects of two key components: estrogen and progestin.[1] Recent advancements in oral contraceptives have focused on developing innovative formulations with fewer side effects and other advantages beyond contraception to ensure increased adherence to contraception methods.[2]

To achieve the balance of effectiveness and safety new formulations has been designed by correctly associating low doses of ethinyl estradiol with drospirenone (DSP). Combination oral contraceptives (COCs) are quite efficient in preventing pregnancy when used consistently.[3,4] The synthetic estrogen ethinylestradiol (EE) is combined with a progestin in the majority of COCs. The EE component has a number of negative effects, one of which is an uncommon but possibly fatal venous thromboembolism.[5,6] The EE dose has been decreased to improve the safety profile of EE-containing COCs. Another option is to substitute natural 17-estradiol for EE, which has a reduced metabolic impact and reduces the risk of thrombosis.[7,8] Estrogen (ESR) is a naturally occurring estrogen that was discovered for the first time in 1965. During pregnancy, only the human fetal liver produces ESR. It exhibits estrogenic actions through binding to nuclear estrogen receptors.[9] Because of its neutral effect on a number of organ tissues, ESR is a promising candidate for usage in COCs. ESR

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is a chemically stable, extremely pure substance made from plant-derived estrone for therapeutic application.[10] ESR 5 mg and 10 mg coupled with DSP 3 mg significantly decreased ovarian activity in a preliminary dose-finding experiment.[11,12]

Several high-performance liquid chromatography (HPLC) methods,[13-16] for quantifying related hormones in various contraceptive formulations have been published in the literature. However, no analytical method for simultaneous quantitative measurement of ESR [Figure 1a] and DSP [Figure 1b] was reported in the literature.

The current method employs RP-HPLC with PDA detection to provide a simple, accurate, and validated analytical approach for the separation and simultaneous estimation of ESR and DSP in oral contraceptives tablets. The method can be used in routine analysis to ensure that these pharmaceutical preparations are of high quality.

MATERIALS AND METHODS

Chromatographic Conditions

Mylan laboratories in Hyderabad, India, provided both drug standards. Sigma-Aldrich provided methanol, water, and acetonitrile (LC grade). SD fine chem, Mumbai, India provided analytical grade sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), and a 0.22 mm membrane filter. Nextstellis tablets containing DSP 3 mg and ESR 14.2 mg were purchased from local pharmacy. All the chemicals used were of the analytical or LC grade. The method was developed using an Agilent LC 1120 with PDA detector and EZ chrome elite software. The two analytes were separated by using Methanol: Phosphate Buffer pH 6.8 adjusted with dil. NaOH (40:60, v/v) as mobile phase pumped at 1.0 ml/min. column temperature was maintained at 30°C and detection wavelength was found at an isosbestic point of 215 nm using a Photo diode array detector. Mobile phase was used a diluent with a total run time of 6 min.

Phosphate Buffer Preparation

To make 1000 mL, dissolved 28.80 g disodium hydrogen phosphate and 11.45 g potassium dihydrogen phosphate with enough water. With dil. NaOH, the pH of the buffer was adjusted to 6.8.

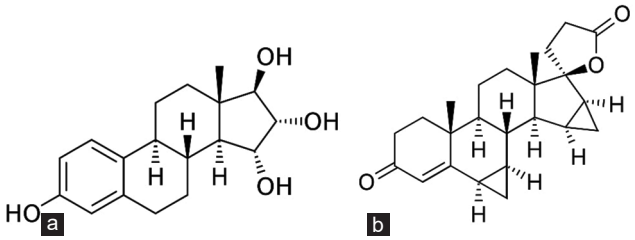

Figure 1: Chemical structure of (a) estetrol; (b) drospirenone

Mobility Phase Preparation

400 mL methanol (40%) was transferred to a 1000 mL volumetric flask, and 600 mL phosphate buffer pH 6.8 (60%) was added, well mixed, and sonicated for 10 min in an ultrasonic water bath. Under vacuum filtration, the fluid was filtered through a 0.45 filter. As a diluent, the mobile phase was utilized.

DSP Working Standard Solutions Preparation

10.0 mg of DSP was accurately weighed and transferred to a 10 mL volumetric flask, dissolved in, and diluted to the mark with the diluent. To produce the final concentration of 80.0 µg/mL, 0.8 mL was removed and diluted to 10 mL with mobile phase.

ESR Working Standard Solutions Preparation

10.0 mg of ESR was accurately weighed and transferred to a 10 mL volumetric flask, dissolved in, and diluted to the mark with the diluent. To achieve the final concentration of 378.0 µg/mL, 3.78 mL was removed and diluted to 10 mL with mobile phase.

Preparation of Sample for Assay Determination

20 DSP and ESR tablets were crushed to powder, and an equivalent to 10 mg of DSP was weighed and transferred to a 10 mL clean and dry volumetric flask. After that, 7 mL of diluent was added and sonicated to thoroughly dissolve it. Finally, using the same solvent, the volume was brought up to the required level (primary formulation stock solution). In addition, 0.8 mL of the above-stock solution was transferred to a standard 10 mL flask and diluted to the mark with the same solvent. To get an 80.0 µg/mL final concentration. The % assay of the formulation was estimated by injecting 10 µL into the HPLC system and measuring the peak area for DSP and ESR.

Validation of the Chromatographic Method

The developed method was validated as per the guidelines of ICH (ICH Guidelines, Q2 (R1), 2005). The method was validated for the following validation characteristics precision, accuracy, linearity, detection and quantification limits, robustness, and forced degradation studies.[17,18]

System Suitability

To validate system performance, system suitability characteristics were assessed. Six injections of standard preparations were used to assess the system's precision. All of the relevant features were measured, including peak area, retention time, tailing factor, peak resolution, and theoretical plate number.

Specificity

The capacity to assess if the analyte of interest can be measured accurately and specifically in the presence of additional components that are likely to be present in the sample is known as specificity. Chromatograms of placebo, commercially available samples, and standard solutions were compared to determine the specificity of the method. The placebo was prepared with the same excipients used in commercial formulations.

Precision

Intraday precision

The intraday precision of the proposed method refers to the ability of the method to reproduce measurements over a short period under the same analytical conditions. To establish intraday precision for the present method, six measurements were made using nominal working concentrations of 80.00 µg/mL for DSP and 378.0 µg/mL for ESR.

Interday precision

Measurements between different days within a laboratory can be reproduced through the proposed method's interday precision. Interday precision was evaluated by injecting samples at nominal working concentrations of 80.00 µg/mL for DSP and 378.0 µg/mL for ESR, on 3 consecutive days.

Precision was expressed as the percentage of relative standard deviation (%RSD) for mean peak area and standard deviation of DSP and ESR.

Accuracy

The proposed assay process measures and expresses accuracy as a percentage of standard analyte recovered from the sample matrix. A known amount of standard is usually added to the sample solution. In triplicate, each concentration level of the analyte was tested at 50, 100, and 150% of the nominal concentration. The proposed method was used to analyze the standards solutions added to the placebo.

Linearity

An analytical procedure can obtain directly proportional results within a specified range about the concentration of the analyte present in the sample. Accordingly, linearity measurements were made using five concentrations ranging from 40 to 120 µg/mL for DSP and from 189 to 567 µg/mL for ESR. Each solution was injected into the chromatographic system in triplicate after being filtered using a 0.45 µm Millipore filter. A plot of concentration versus mean area was used to evaluate the analytical curve. A least-squares regression procedure was used to obtain the equation.

Detection and Quantification Limits

The detection and quantitative limits for the analytical method have been calculated based on the residual standard deviation of the regression line (σ) and slope (S) of the analytical curve based on DL = 3.3 (σ/S) and QL = 10 (σ/S).

Robustness

The ability of a methodology to remain unaffected by slight changes in parameters is known as method robustness (ICH 2005). Three chromatographic parameters were employed to determine robustness in this study: Flow rate (1.00 mL/min) and change in mobile phase organic content (5%). The resolution between peaks parameter was tested 3 times, above (+) and below (-) the nominal value.

Forced Degradation Studies

Stress testing is required by the ICH Guidelines, Q1A R2, 2005 guideline[18] stability testing of novel drug substances and products to define the intrinsic stability properties of the active ingredient. The purpose of this study was to use the proposed approach to conduct stress degradation experiments on the DSP and ESR.

Acidic and Alkaline Hydrolysis

Transferred 0.80 mL of the primary stock solution to two 10 mL standard flasks. For acidic conditions, 5 mL of 1 N HCl was added to the aforesaid solution in one pair of 10 mL standard flasks. In another set of 10 mL standard flasks, 3 mL of 1 N NaOH was added for alkaline degradation. After that, the acid and alkaline samples were maintained in a water bath at 65°C for 6 h and 75°C for 5 h, respectively. Both sets of solutions were neutralized and diluted to 10 mL with diluent, yielding 80 µg/mL for DSP and 378 µg/mL for ESR, respectively. Allowed the solution to cool at room temperature. Filtered the solution with a 0.22 mm syringe before injecting it into the HPLC system vials.

Oxidative Degradation

Filled a 10 mL standard with 0.80 mL of main stock solution. The volume was brought up to the mark using diluents and 3 mL of 6 % (w/v) hydrogen peroxide was added to achieve 80 µg/mL and 378 µg/mL for DSP and ESR, respectively. The standard flask was preheated for 5 h at 75°C. After filtering using a 0.22 mm syringe filter, the resultant solution was cooled and put into the HPLC system vials.

Thermally Induced Degradation

Transferred 0.80 mL of main stock solution to a 10 mL standard flask and dilute to the desired amount with diluent to

get DSP and ESR concentrations of 80 µg/mL and 378 µg/mL, respectively. The solution was then refluxed for 3 h at 85°C. The solution was then brought at room temperature. After filtering with a 0.22 mm syringe filter, pour into the HPLC system vials.

Photodegradation

Pipetted 0.80 mL from the stock solution into a 10 mL standard flask and fill to the mark with diluent to achieve 80 µg/mL for DSP and 378 µg/mL for ESR, respectively. The samples were then transferred to a Petri plate and placed in a photostability chamber for 24 h at 200 Wh/m2 UV light and 1.2 million Lux hours UV light. Bought the finished product to room temperature. Filtered the solution with a 0.22 mm syringe before injecting it into the HPLC system vials.

RESULTS AND DISCUSSION

Method Validation

Method validation, as defined by ICH, entails testing an analytical method to ensure that it is accurate, specific, and reproducible over the range of concentrations and within the analytical conditions described. The developed method was validated as per the guidelines of ICH (ICH Guidelines, Q2 (R1), 2005).

Table 1: System suitability results

Parameter*	DSP	EST
Theoretical Plate Count	6402	4025
Average Peak Area	2138271	12514807
Peak Height	243212	2169079
RT	4.602	2.391
Tailing	1.01	1.21
Resolution	-	11.32
S/N	1669.82	464.25

* Average of 6 replicates

System Suitability Study

System suitability was attained by checking various parameters and found within the ICH limit. The results are presented in Table 1. Figure 2 (a-c) shows the representative chromatograms of (a) Blank, (b) standard and (c) sample of DSP and ESR.

Specificity

During the analysis of DSP and ESR, there was no interference, demonstrating the specificity of the method. This method does not exhibit any interference by excipients. Therefore, there is no overlap between DSP and ESR peaks. As shown in Figure 2b and c, despite the presence of peaks for components of pharmaceutical formulations (excipients), DSP and ESP peaks were separated adequately.

Accuracy

The recovery study was used to determine the accuracy and the results are presented in Table 2. Recovery of the DSP standard was 99.93%, and for the ESR standard recovery was 99.87%. Based on the obtained results, the method seems to be accurate.

Precision

Analytical precision provides insight into method random error. DSP and ESR reproducibility and intermediate precision were estimated. For DSP and ESR, the % RSD was 0.60 and 0.71%, respectively, (reproducibility precision). %RSD < 2.0% is an essential requirement for both reproducibility and intermediate precision. Analyzing sample solution of dosage forms at optimized concentrations on 3 consecutive days at six replicates each allowed us to obtain the %RSD intermediate precision. The coefficients of variation were 0.63 and 0.55% for DSP and ESR, respectively. This indicates that the developed method has good precision. Table 3 shows the results obtained from the analysis of commercially available samples.

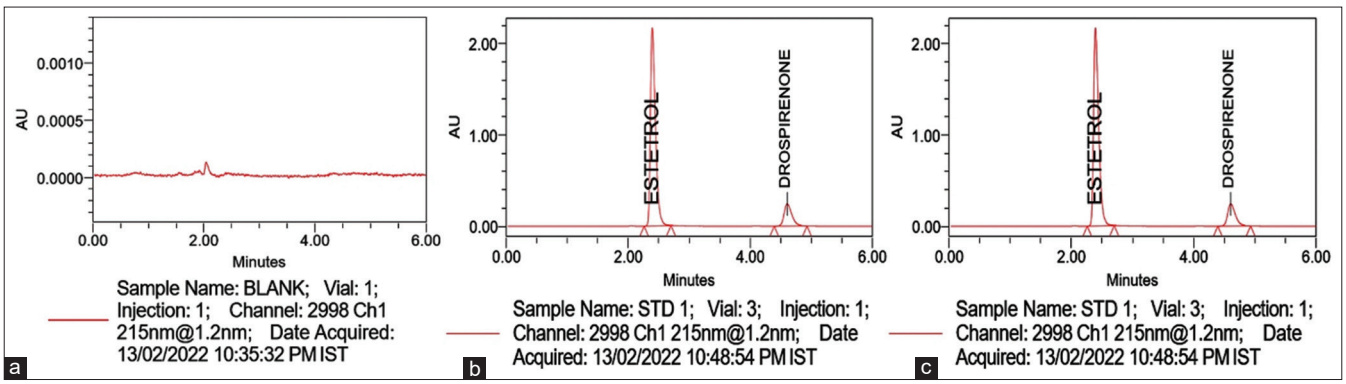

Figure 2: Representative chromatograms of (a) Blank; (b) Standard; (c) Sample

Table 2: Results of accuracy

		DSP				
Accuracy Level	**Wt. of Sample (mg)**	**Peak Area**	**Amount Added (µg/mL)**	**Amount Found (µg/mL)**	**% Recovery**	**Mean % Recovery**
50%	153.67	1071499	39.88	40.09	100.52	99.93
100%	307.33	2127492	79.76	79.60	99.80	
150%	461.00	3181121	119.64	119.02	99.48	
ESTERTROL						
50%	153.67	6209103	187.72	187.54	99.90	99.87
100%	307.33	12465891	375.44	376.52	100.29	
150%	461.00	18535757	563.17	559.86	99.41	

Table 3: Precision results

S. No	**Reproducibility**		**Intermediate Precision**	
	Peak Area			
	DSP	**EST**	**DSP**	**EST**
1	2145265	12523307	2132304	12508045
2	2142757	12536111	2140220	12521643
3	2143529	12529869	2115333	12372333
4	2139243	12557166	2117149	12372949
5	2113402	12419708	2136308	12381516
6	2124839	12553232	2149651	12424701
Average	2134839.17	12519898.83	2131827.50	12430197.83
STDEV	12851.87	50817.32	13386.11	68481.10
% RSD	0.60	0.41	0.63	0.55

Table 4: Robustness results

Parameter	**Condition**	**DROSPIRENONE**			**ESTERTROL**		
		RT	**Peak Area**	**% Assay**	**RT**	**Peak Area**	**% Assay**
Flow	0.8 ml/min	6.098	2127984	99.52	3.181	12437314	99.21
	1.0 ml/min	4.602	2138271	100.00	2.391	12514807	99.83
	1.2 ml/min	3.753	2134138	99.81	1.922	12457028	99.36
M P MeoH: Phosphate Buffer (40:60, v/v)	35:65 v/v	4.663	2124377	99.35	2.394	12556345	100.16
	40:60 v/v	4.602	2138271	100.00	2.391	12514807	99.83
	45:55 v/v	4.642	2129427	99.59	2.403	12481162	99.56

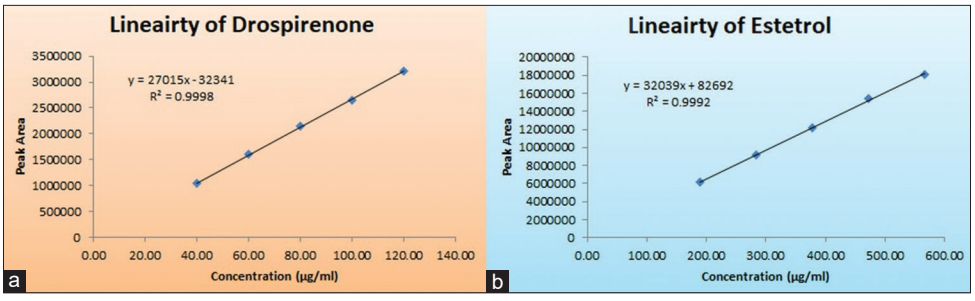

Figure 3: Linearity curves of (a) Drospirenone; (b) Estertrol

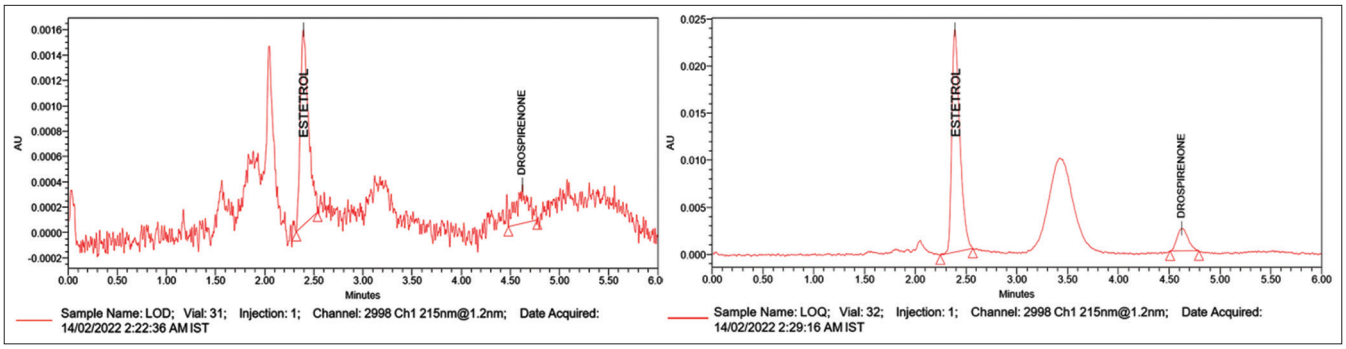

Figure 4: Chromatograms of LOD and LOQ

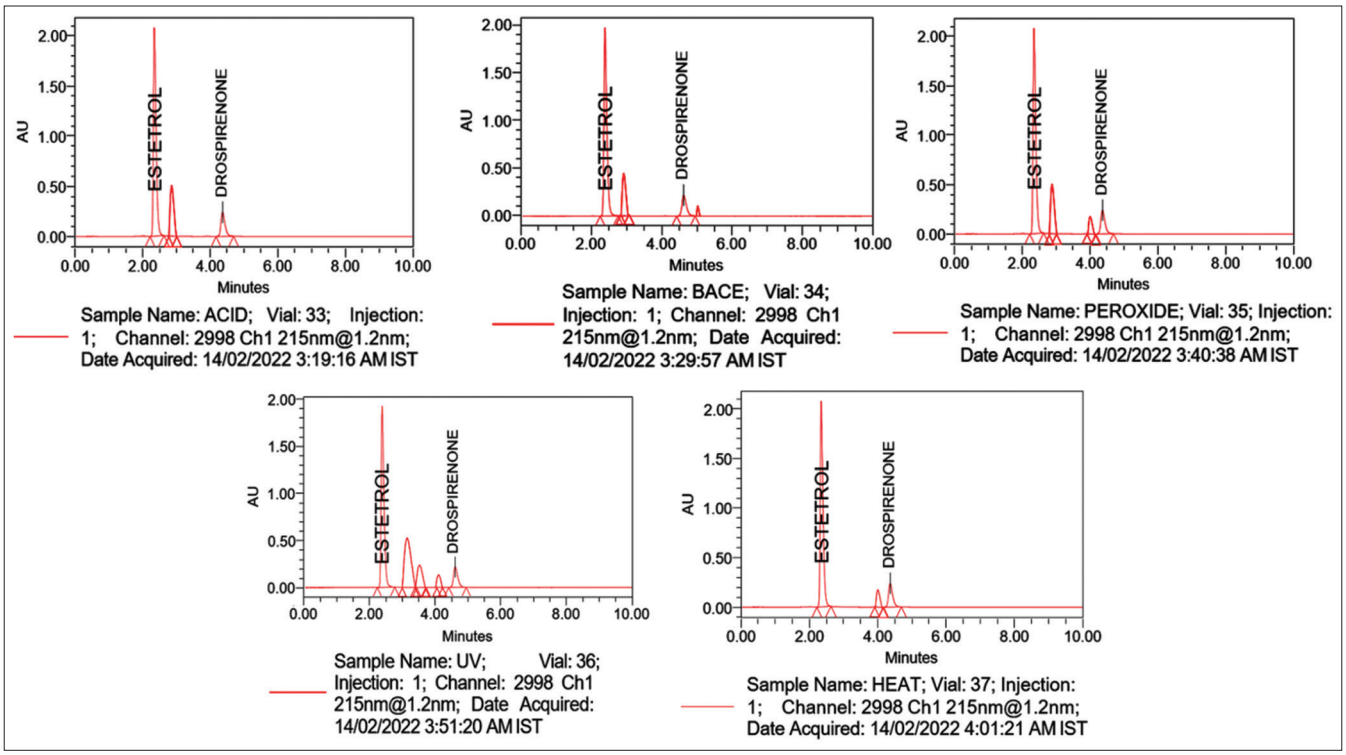

Figure 5: Degradation chromatograms

Table 5: Results of Assay

S. No	Peak Area		% Assay	
	DSP	EST	DSP	EST
1	2145265	12523307	100.33	99.89
2	2142757	12536111	100.21	99.99
3	2143529	12529869	100.25	99.95
4	2139243	12557166	100.05	100.16
5	2113402	12419708	98.84	99.07
6	2124839	12553232	99.37	100.13
Average	2134839.17	12519898.83	99.84	99.87
STDEV	12851.87	50817.32	0.60	0.41
% RSD	0.60	0.41	0.60	0.41

Linearity

Linearity was built using five concentrations ranging from 50 to 150 % (40.00, 60.00, 80.00, 100.00, and 120.00 µg/mLDSP and 189.00, 283.50, 378.00, 472.50, and 567.00 µg/mL ESR). In the examined concentration range, the analytes' peak areas were found to be linear, with a correlation coefficient of greater than 0.999 for both analytes. Figure 3 depicts the linearity data and curve (a and b).

Detection and Quantification Limits

LOD and LOQ were determined by using standard deviation of response and slope of calibration curves. The LOD and

Table 6: Results of degradation studies

Degradation	DROSPIRENONE			ESTERTROL		
	Peak Area	% Assay	% Deg	Peak Area	% Assay	% Deg
Acid	1906340	89.15	10.85	11284887	90.01	9.99
Base	1918103	89.70	10.30	11337957	90.44	9.56
Peroxide	1930370	90.28	9.72	11325314	90.34	9.66
Thermal	1933375	90.42	9.58	11459815	91.41	8.59
Photo	1922047	89.89	10.11	11301993	90.15	9.85

LOQ were found to be 0.28 and 0.95 µg/mL for DSP and 0.76 and 2.53 µg/mL for EST, respectively. Figure 4 shows the chromatograms of LOD and LOQ.

Robustness

The robustness of the analytical method was assessed by analyzing the test solutions after making small but purposeful modifications to the parameters such as flow rate (±0.1 mL/min) and mobile phase concentration (±5%). During various analytical settings, the data on system applicability were determined to be adequate. Slight adjustments in the analytical conditions had no effect on the system suitability results. The findings of DSP and ESR robustness are shown in Table 4.

Assay

The suggested methodology was used to test DSP and ESR tablets, and the mean percent assay for DSP and ESR was determined to be 99.84 and 99.87%, respectively. Table 5 shows the results.

Forced Degradation Studies

By subjecting the sample solution to various stress conditions such as acid, base, peroxide, UV, and heat, forced degradation tests were conducted to assess the stability indicating ability of the suggested analytical technique. Stress samples were tested against a reference standard in assay studies. The suggested analytical technique can identify the analyte even in the presence of deteriorated products, demonstrating the created method's stability. Table 6 displays the results of the stress investigations, and Figure 5 shows the chromatograms of the DSP and ESR stress studies.

CONCLUSION

For the simultaneous estimation of DSP and ESR in tablet formulations, a simple, accurate, and precise stability-indicating RP-HPLC analytical method was designed and validated. This method's low LOD and LOQ allow for detection and measurement of the analytes at low concentrations. Because both peaks are widely separated from one another and excipients with a total run time of 6 min, the approach is relatively simple and specific, making it ideal for routine quality control analytical work.

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