Journal of
Analytical & Bioanalytical Techniques

Chandran et al., J Anal Bioanal Tech 2018, 9:4
DOI: 10.4172/2155-9872.1000408

Research Article Open Access

Simultaneous Quantification of Drospirenone, Ethinyl Estradiol and Levomefolate by Stability Indicating RP-HPLC Method

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Abstract

A new sensitive, selective, precise and accurate stability indicating reverse phase high performance liquid chromatographic method has been developed for the simultaneous quantification of drospirenone, ethinyl estradiol and levomefolate in bulk and combined tablet dosage form. Separation and analysis of drospirenone, ethinyl estradiol and levomefolate was achieved on Waters C18 (5 µm, 250 mm × 4.6 mm) column using 0.1% H_3PO_4, methanol and acetonitrile in the ratio of 60:20:20 (*v/v/v*) as mobile phase at 27°C. The flow rate was 1.0 mL/min. The effluents were monitored with detector set at 245 nm. The method validation was done with regard to the guidelines by the International Conference on Harmonization and US Food and Drug Administration. All the validation characteristics are within the acceptance criteria. The studied drugs were subjected to acid, alkali and neutral hydrolysis, hydrogen peroxide oxidation, thermal degradation, and photo (sunlight) degradation. The peaks of degradation products were well resolved from the peaks of three analytes (drospirenone, ethinyl estradiol and levomefolate). Hence, the developed and validated liquid chromatographic method is able to quantify the drospirenone, ethinyl estradiol and levomefolate in the presence of degradation products.

Keywords: Drospirenone; Ethinyl estradiol; Levomefolate; Safyral; HPLC; Quantification

Introduction

Drospirenone is a synthetic progestogen, progestin, with anti-mineralocorticoid and progestational activity [1]. Drospirenone is used in menopausal hormone therapy, treatment of premenstrual dysphoric disorder and acne [2-4]. Drospirenone is also an important ingredient in most of the oral contraceptive pills. Chemically, drospirenone is described as (6R,7R,8R,9S,10R,13S,14S,15S,16S,17S)-1,3',4',6,6a,7,8,9,10,11,12,13,14,15,15a,16-Hexadecahydro-10,13-dimetylspiro-[17H-dicyclopropa[6,7:15,16]cyclopenta[a]phenantrene-17,2'(5'H)-furan]-3,5'(2H)-dione (Figure 1). Drospirenone exerts its activity through binding strongly and specifically to progesterone receptor [5,6]. Drospirenone-progesterone receptor complex produces an activated complex which binds to specific sites in DNA. As the result, luteinizing hormone activity is suppressed and ovulation is inhibited. This activated complex also changes the cervical membrane and endometrium.

Ethinyl estradiol, semisynthetic estrogen, is an estrogen receptor agonist [7]. Chemically, ethinyl estradiol is known as (8R,9S,13S,14S,17R)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (Figure 1). Ethinyl estradiol, alone is used in post menopausal hormonal replacement therapy, and to treat female hypogonadism, and symptoms of breast cancer and prostate cancer. Ethinyl estradiol is also used as oral contraceptive in combination with progestin [8,9]. The complex formed through the binding of ethinyl estradiol to estrogen receptor increases the transcription of genes which are responsible for estrogenic cellular responses [10]. By inhibiting 5-α-reductase enzyme, ethinyl estradiol lessens testosterone levels and disrupts the prostatic cancer progression [11].

Levomefolate, chemically known as (2S)-2-[[4-[(2-Amino-5-methyl-4-oxo-1,6,7,8-tetrahydropteridin-6-yl)methylamino]benzoyl]amino]pentanedioic acid (Figure 1), is an biologically active of vitamin B9 (folic acid) [12]. Levomefolate plays an important role in synthesis of DNA, cysteine cycle and regulation of metabolism of homocysteine. Levomefolic acid is prescribed for patients with symptoms of vitamin B12 deficiency [13]. Levomefolate is also been used for treating patients with cardiovascular disease and cancers of breast and colorectal [14,15].

Drospirenone, ethinyl estradiol and levomefolate combination is available in oral contraception tablet dosage form with brand names Safyral, Beyaz and Rajani [16-19]. In this combination, drospirenone and ethinyl estradiol prevent pregnancy by repressing ovulation. These two drugs also make changes in cervical mucus and endometrial which inhibits penetration of sperm and lessen the implantation, respectively. Levomefolate in the tablet increases the levels of folate levels in women who opt oral contraceptive [20].

To the best of our knowledge, there is no report for the simultaneous determination of drospirenone, ethinyl estradiol and levomefolate in bulk and combined tablet dosage form by stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method. Therefore, the main aim of this investigation was to develop and validate a stability indicating RP-HPLC method to determine drospirenone, ethinyl estradiol and levomefolate simultaneously in the presence of their stress degradation products [21-23].

Experimental

Materials

Drospirenone, ethinyl estradiol and levomefolate reference drug standards were provided kindly by Rainbow Pharma Training Labs (Hyderabad, India). Safyral (Bayer Health Care Pharmaceuticals Inc.

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Received June 25, 2018; **Accepted** July 09, 2018; **Published** July 15, 2018

Citation: Chandran S, Xavier Rajarathinam SR, Kalaiselvan A (2018) Simultaneous Quantification of Drospirenone, Ethinyl Estradiol and Levomefolate by Stability Indicating RP-HPLC Method. J Anal Bioanal Tech 9: 408. doi: 10.4172/2155-9872.1000408

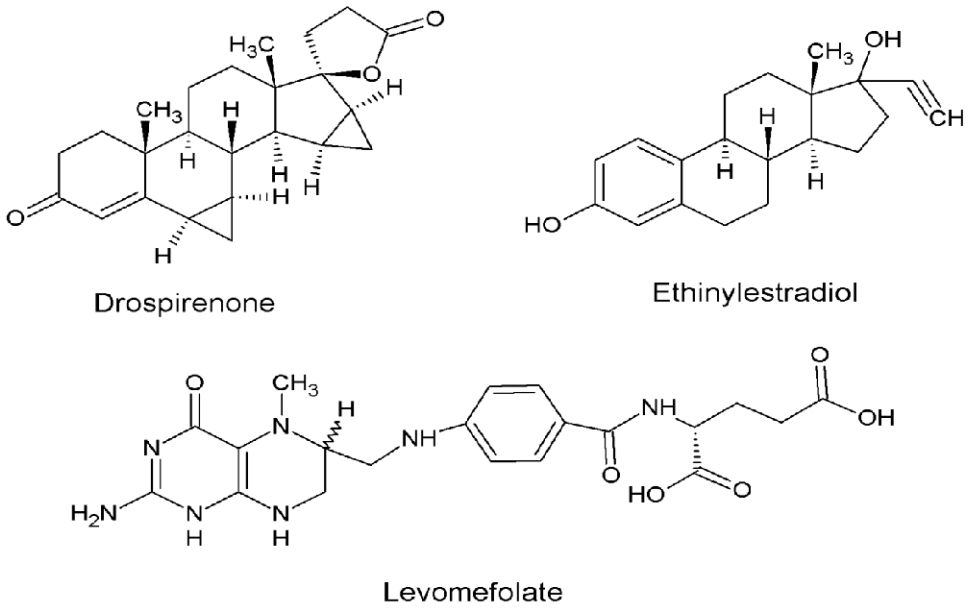

Figure 1: Structures of studied drugs.

Whippany, NJ) tablets labeled to contain 3 mg drospirenone, 0.03 mg ethinyl estradiol and 0.451 mg levomefolate were obtained from a local pharmacy market. HPLC grade acetonitrile and methanol were purchased from Merck India Ltd (Mumbai, India). Analytical reagent orthophosphoric acid, hydrogen peroxide, hydrochloric acid and sodium hydroxide were supplied by Sd. Fine Chemicals Ltd (Mumbai, India). HPLC grade water was prepared using Milli-Q system (Millipore, USA).

Instrumentation

Waters Alliance HPLC system 2695 Module with a 2998 PDA detector, degasser, auto sample injector and column oven were used in the present analysis. Data acquisition and processing was done with Empower 2 software. Method development and validation was done using Waters, C18, 5 µm, 250 mm × 4.6 mm analytical column.

Optimized HPLC conditions

Isocratic elution was performed with a mobile phase comprised of filtered (using a 0.45 µm membrane filter) and degassed 0.1% orthophosphoric acid: methanol: acetonitrile (60:20:20 *v/v/v*) adjusted to pH 4.8 and pumped at a flow rate of 1.0 mL/min. The column temperature was set at 27°C. The samples were injected at 10 µL injection volume and eluted samples were analyzed at a wavelength of 245 nm. The total runtime was 8 min.

Standard stock and working solutions

The standard stock solution (Drospirenone–1200 µg/mL, Ethinyl estradiol–12 µg/mL and Levomefolate–180.4 µg/mL) was prepared by dissolving an accurately weighed 30 mg, 0.30 mg and 4.51 mg of drospirenone, ethinyl estradiol and levomefolate, reference standard respectively in 25 mL of mobile phase in a volumetric flask (25 mL). The working standard solutions in the range 30–240 µg/mL of drospirenone, 0.3–2.4 µg/mL of ethinyl estradiol and 4.51–36.08 µg/mL of levomefolate were obtained by appropriately diluting the standard stock solution with mobile phase.

Construction of calibration curve

Aliquots (10 µL) of working standard solutions were injected into the HPLC system and eluted by the mobile phase under the optimum HPLC conditions. The peak area response of drug versus the final concentration of drug (µg/mL) was plotted. On the other hand, the corresponding regression equations were derived.

Analysis of drospirenone, ethinyl estradiol and levomefolate in tablet sample solution

Ten tablets were crushed into powder. The tablet powder weight equivalent to 30 mg, 0.30 mg and 4.51 mg of drospirenone, ethinyl estradiol and levomefolate, respectively was transferred to 25 mL volumetric flask and sonicated with 10 mL of mobile phase for 20 min. The volume was diluted to 25 mL with mobile phase and filtered through 0.45 µm membrane filter. The stock tablet sample solution was then diluted aptly with mobile phase to get the final concentration 120 µg/mL, 1.2 µg/mL and 18.04 µg/mL of drospirenone, ethinyl estradiol and levomefolate, respectively. 10 µL of working tablet sample solution prepared was injected into the HPLC system and analyzed by the developed method. The nominal content of drospirenone, ethinyl estradiol and levomefolate in the tablet was calculated either using the corresponding calibration graph or corresponding regression equation.

Stress degradation studies

The stress degradation studies were performed through the analysis of tablet sample solution (drospirenone-120 µg/mL, ethinyl estradiol-1.2 µg/mL and levomefolate -18.04 µg/mL), which was exposed to accelerated degradation conditions as per the ICH guidelines [21]. The results are compared to a reference standard solution prepared in the same day.

Acid and alkaline hydrolysis

Volumetric flasks (100 mL) containing 10 mL of tablet sample solution (drospirenone–1200 µg/mL, ethinyl estradiol–12 µg/mL and levomefolate–180.4 µg/mL) were mixed with 10 mL of 0.1 N

HCl solution for acidic degradation acid or 10 mL of 0.1 N NaOH solution for alkaline degradation. The solutions were sonicated at room temperature for 30 min. After this period, the acid and alkali degraded solutions were neutralized with apt volume of 0.1 N NaOH and 0.1N HCl, respectively. The resulting solutions were diluted with mobile phase to get a concentration of 120 µg/mL, 1.2 µg/mL and 18.04 µg/mL drospirenone, ethinyl estradiol and levomefolate, respectively. The solutions were filtered and injected.

Thermal and photo degradation

10 mL of tablet sample solution (drospirenone–1200 µg/mL, ethinyl estradiol–12 µg/mL and levomefolate–180.4 µg/mL) was transferred to volumetric flask (100 mL) and exposed to 105°C for 30 min in oven (for thermal degradation) or exposed to sun light for 24 hr (for photo degradation). After the specified period of degradation, the resulting solution was diluted with mobile phase for a concentration of 120 µg/mL, 1.2 µg/mL and 18.04 µg/mL drospirenone, ethinyl estradiol and levomefolate, respectively. The solutions were filtered and injected.

Oxidative and neutral degradation

10 mL of 30% hydrogen peroxide solution (for oxidative degradation) or 10 mL of deionised water (for neutral degradation) was added into a 100 mL volumetric flask containing 10 mL tablet sample solution (drospirenone–1200 µg/mL, ethinyl estradiol–12 µg/mL and levomefolate–180.4 µg/mL). After sonication for 30 min at room temperature, the solutions were diluted to 100 mL with mobile phase until a concentration 120 µg/mL, 1.2 µg/mL and 18.04 µg/mL of drospirenone, ethinyl estradiol and levomefolate, respectively was obtained. These solutions were filtered and injected.

Results and Discussion

Optimization of HPLC conditions

Preliminary studies involved testing different mobile phase compositions, pH, flow rates, temperatures and detection wavelength for the effective separation and simultaneous analysis of drospirenone, ethinyl estradiol and levomefolate. Trial experiments were conducted using a Waters C18 column with a length of 250 mm, internal diameter of 4.6 mm and particle size of 5 µm as the stationary phase. Different proportions of 0.1% orthophosphoric acid, acetonitrile and methanol with different pH units in isocratic elution mode were investigated to obtain optimum resolution, symmetric peak shape and optimal sensitivity in reasonable time. Best results were obtained with a mixture of 0.1% orthophosphoric acid, acetonitrile and methanol in the ratio of 60:20:20 (*v/v/v*) with pH 4.8 units was employed as the mobile phase. The flow rate of mobile phase, for improved resolution and quick separation, was adjusted to 1.0 mL/min. Room temperature was adequate for the separation and analysis of selected drug combination and so the same was used in the whole separation and analysis. Detection at 245 nm was used as it was observed as the optimum detection wavelength for the three analytes (drospirenone, ethinyl estradiol and levomefolate). At this detection wavelength (245 nm), the peak area response for the three analytes was high. Representative chromatogram of the finalized chromatographic conditions, showing drospirenone, ethinyl estradiol and levomefolate, is illustrated in Figure 2.

Method validation

The method was validated following ICH and FDA guidelines for system suitability, selectivity, specificity, linearity, sensitivity, accuracy, precision and robustness [22,23].

System suitability

System suitability parameters like peak tailing, plate count, resolution, and percent relative standard deviation for retention time and peak area response were calculated to demonstrate that the HPLC system performed well. For this study, standard solution (drospirenone-120 µg/mL, ethinyl estradiol-1.2 µg/mL and levomefolate-18.04 µg/mL) was injected into the HPLC system in five replicates. The obtained values were in the acceptable limits as given in Table 1.

Selectivity

The selectivity of the method was evaluated by comparison of chromatograms of blank mobile phase, placebo blank (mixture of excipients), tablet sample solution with standard solution. The representative chromatograms of the four samples are shown in Figure 3a-3d. The chromatograms of blank mobile phase (Figure 3a) and placebo (Figure 3b) did not show a response at the retention times of three analytes. Interfering peaks are not found in the chromatogram of the tablet sample (Figure 3d), demonstrating that excipients used

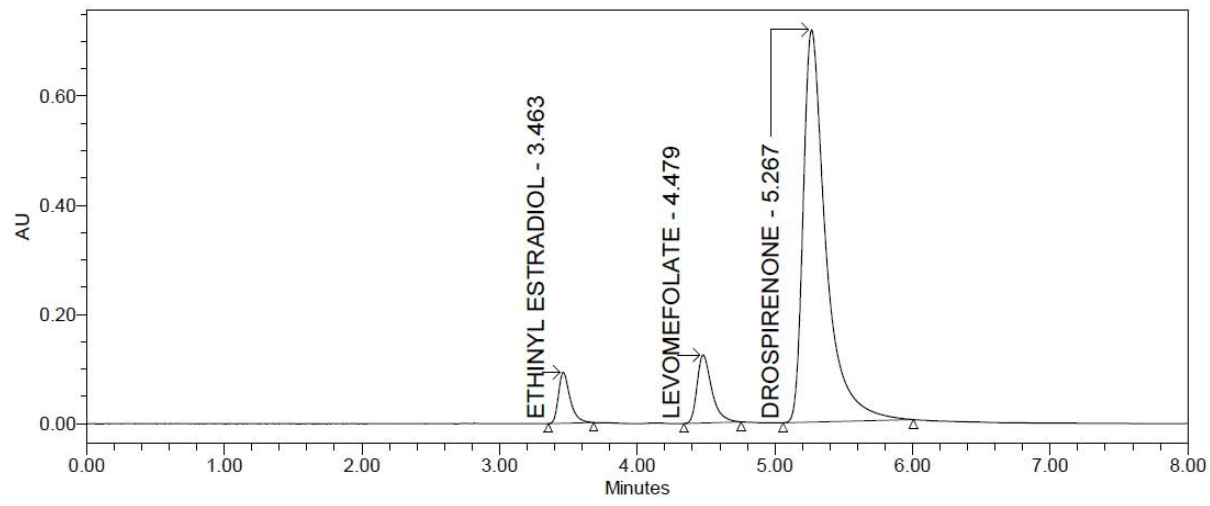

Figure 2: Chromatogram of well separated peaks of drospirenone, ethinyl estradiol and levomefolate.

Drug / Parameter	Ethinyl estradiol		Levomefolate		Drospirenone		Recommended limit
	Value*	RSD (%)	Value*	RSD (%)	Value*	RSD (%)	
RT** (min)	3.472	0.232	4.485	0.169	5.268	0.175	RSD ≤ 2
Peak area (mAU)	548342	0.439	924440	0.506	8225662	0.437	RSD ≤ 2
Plate Count	8501	0.645	9101	0.353	5773	0.948	>2000
Peak Tailing	1.414	0.387	1.436	0.381	1.756	0.312	≤ 2
Resolution	-	-	5.794	0.262	3.238	0.594	>1.5

Table 1: System suitability parameters of the method for drospirenone, ethinyl estradiol and levomefolate analysis.

*Average of five determinations; **Retention time

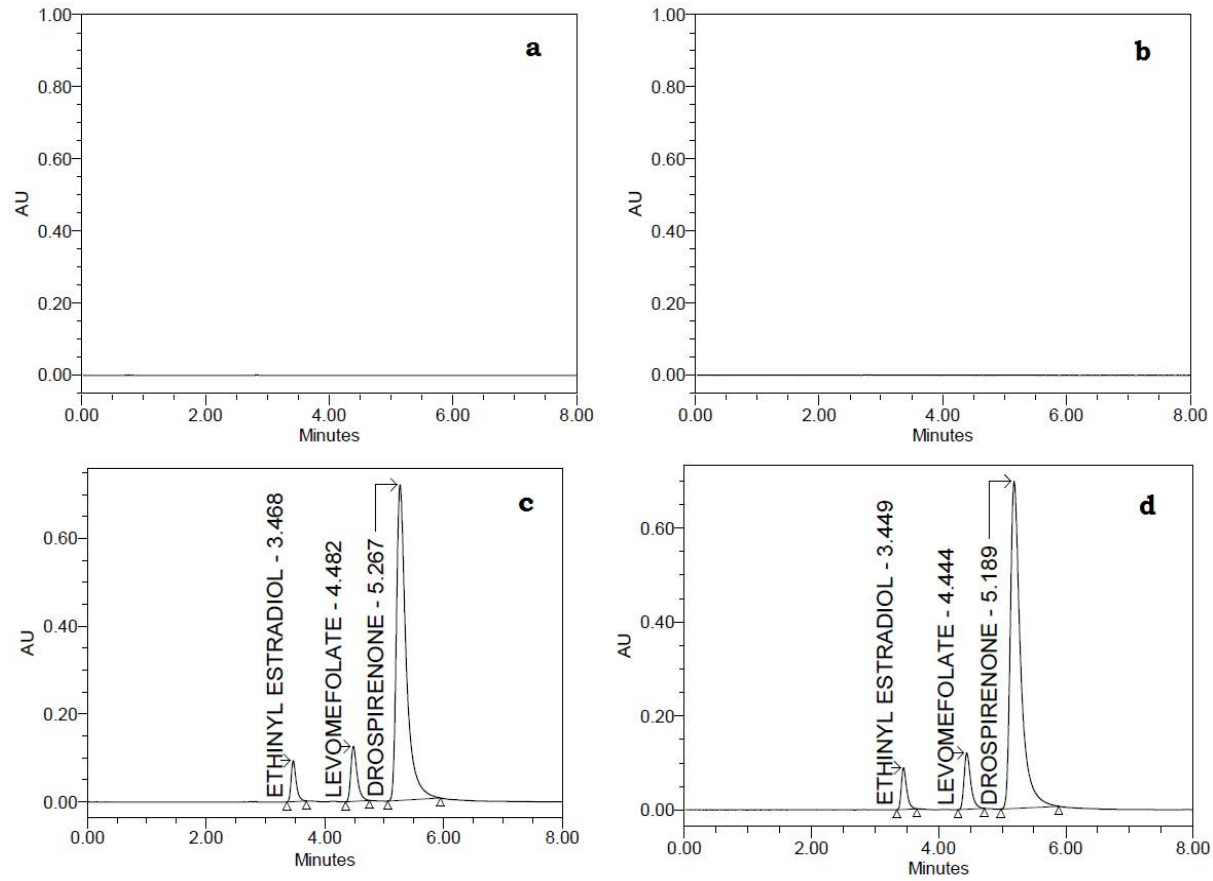

Figure 3: HPLC chromatograms of solutions of [a] Blank mobile phase [b] Placebo blank [c] Standard (drospirenone-120 µg/mL, ethinyl estradiol-1.2 µg/mL and levomefolate-18.04 µg/mL) [d] Tablet sample (drospirenone-120 µg/mL, ethinyl estradiol-1.2 µg/mL and levomefolate-18.04 µg/mL).

in the tablets did not interfere with the peaks of drospirenone, ethinyl estradiol and levomefolate. This proved the method selectivity.

Specificity

Stress degradation was done to demonstrate the method specificity, stability of the drugs, detect the possible degradation products and stability indicating properties of the developed method. Stress degradation was carried out by exposing tablet sample solution to stress conditions of hydrolysis (acid, alkali and neutral), oxidation, photo and thermal. Stressed samples were analyzed by the proposed method. The corresponding peaks were checked for the peaks interference, retention times, and peak purity. The percentage of degradation in all the stressed samples was also determined.

The chromatograms of acid, base, hydrogen peroxide, heat, sun light and water degraded tablet sample solution are shown in Figure 4a-4e. The chromatograms showed no interference between the peaks of studied drugs (levomefolate, ethinyl estradiol, drospirenone) and the degradation product produced in the applied stress conditions. The percentage of recovery and degradation results of the forced degradation studies are summarized in Table 2. The applied stress conditions were enough to degrade the three drugs. The percent degradation value comparison of the three drugs showed that the order of stability is: levomefolate>ethinyl estradiol>drospirenone. The degradation product was observed at retention times of 2.882 min (acid hydrolysis, Figure 4a), 2.870 min (base hydrolysis, Figure 4b), 2.876 min (oxidative degradation, Figure 4c), 2.867 min (thermal degradation, Figure 4d), 2.866 min (photo degradation, Figure 4e) and 2.864 min (neutral hydrolysis, Figure 4f). The peak of degradation product is well resolved from the analytes peaks using the proposed method. The homogeneity of the peaks of studied drugs was checked using photodiode array detector. The results were shown in Table 2.

Analyte	Degradation condition	Peak area (mAU)	Percent of drug		Peak purity	
			Recovered (%)	Degraded (%)	Purity angle	Purity threshold
Ethinyl Estradiol	Undegraded	548342	99.60	-	-	-
	Acidic	477576	86.75	13.25	0.344	0.959
	Basic	472362	85.80	14.20	0.300	0.871
	Oxidative	463459	84.18	15.82	0.355	0.860
	Thermal	482790	87.69	12.31	0.388	0.764
	Photo	494754	89.87	10.13	0.341	0.861
	Neutral	493323	89.61	10.39	0.379	0.863
Levomefolate	Undegraded	924439.7	99.50	-	-	-
	Acidic	829896	89.32	10.68	0.249	0.800
	Basic	836466	90.03	9.97	0.422	0.904
	Oxidative	833293	89.69	10.31	0.382	0.693
	Thermal	824163	88.71	11.29	0.298	0.596
	Photo	822654	88.54	11.46	0.378	0.694
	Neutral	831421	89.49	10.51	0.320	0.699
Drospirenone	Undegraded	8225662	99.60	-	-	-
	Acidic	6980365	84.52	15.48	0.269	0.883
	Basic	7193782	87.11	12.89	0.231	0.681
	Oxidative	6880465	83.31	16.69	0.239	0.578
	Thermal	6730682	81.5	18.50	0.241	0.579
	Photo	7246081	87.74	12.26	0.236	0.579
	Neutral	7323993	88.68	11.32	0.243	0.580

Table 2: Stress degradation results of drospirenone, ethinyl estradiol and levomefolate in tablet sample solution.

The increased peak threshold value than peak purity angle value (Table 2) confirmed the purity and homogeneity of levomefolate, ethinyl estradiol, drospirenone peaks in all the stress conditions applied. The results of stress degradation studies proved the specificity and stability indicating properties of the developed HPLC method.

Linearity

The calibration curve was constructed by plotting the peak area response (mAU) of drug against the concentration (µg/mL). Calibration curve was linear over a range of concentration 0.3-2.4 µg/mL (ethinyl estradiol), 4.51-36.08 µg/mL (levomefolate) and 30-240 µg/mL (drospirenone). Linear regression equation and regression coefficient (R^2) were:

y=45651 x+670.3 and 0.9996, respectively for ethinyl estradiol.

y=51146 x+673.7 and 0.9999, respectively for levomefolate

y=68231 x+10671 and 0.9998, respectively for drospirenone

where 'y' is peak area response (mAU) and 'x' is concentration of drug (µg/mL). The results showed excellent correlation exists between the peak area response and concentration.

Sensitivity

The method sensitivity was determined with respect to limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were assessed at a signal-to-noise ratio of 3:1 and 10:1, respectively using the developed method by analyzing different dilute solutions of drospirenone, ethinyl estradiol and levomefolate. The determined LOD values are 0.010 µg/mL, 0.109 µg/mL and 0.126 µg/mL for ethinyl estradiol, levomefolate and drospirenone, respectively. The determined LOQ values are 0.032 µg/mL, 0.363 µg/mL and 0.420 µg/mL for ethinyl estradiol, levomefolate and drospirenone, respectively. The values showed that the sensitivity of the method was good.

Precision

The precision of the HPLC method for drospirenone, ethinyl estradiol and levomefolate was evaluated by analyzing standard solution (drospirenone-120 µg/mL, ethinyl estradiol-1.2 µg/mL and levomefolate-18.04 µg/mL) six times. Percentage relative standard deviation (%RSD) of peak area response of the studied drugs was used to assess the precision. The results of precision exhibited %RSD below 0.5% (Table 3), indicating the excellent precision of the method.

Accuracy

The method accuracy for drospirenone, ethinyl estradiol and levomefolate was determined by analyzing standard solution (drospirenone-120 µg/mL, ethinyl estradiol-1.2 µg/mL and levomefolate-18.04 µg/mL) six times. The accuracy of the results was demonstrated by calculating the percent recovery. The results showed good accuracy performance for the determination of the three analytes (Table 4).

Recovery

The newly developed HPLC method was further evaluated for its accuracy by the analysis of the placebo spiked with pure drospirenone, ethinyl estradiol and levomefolate at three different concentration levels. Recovery of the spiked drospirenone, ethinyl estradiol and levomefolate was determined by the proposed method three times. The recovery values (Table 5), indicating that the developed method ensure the acquisition of reliable accurate data for drospirenone, ethinyl estradiol and levomefolate at different concentrations.

Robustness

Method robustness was established by deliberately varying the experimental conditions such as flow rate (± 0.1 mL/min), column oven temperature (± 2°C), mobile phase components ratio (± 5%), pH of mobile phase (±0.2 units) and detection wavelength (±2 nm). The study was carried out on the same day with standard solution

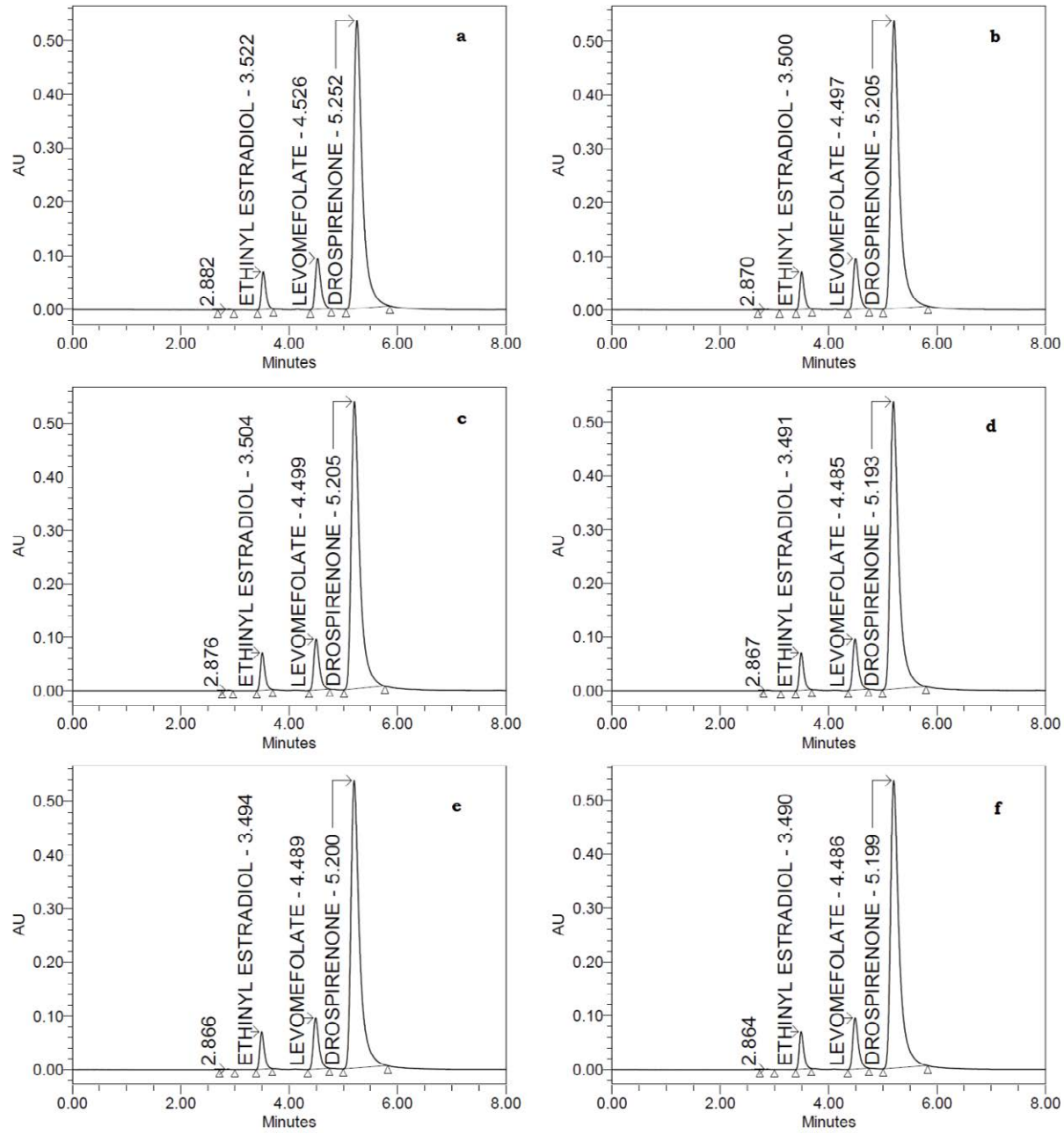

Figure 4: Chromatograms from 'a' to 'f' are samples that have been subjected to acid hydrolysis, alkali hydrolysis, oxidative degradation, thermal degradation, photo degradation and neutral hydrolysis, respectively.

Injection No.	Peak area response of drug (mAU)		
	Ethinyl estradiol	Levomefolate	Drospirenone
1	548672	924625	8223258
2	548654	924095	8227782
3	548533	924445	8229372
4	548370	924665	8220001
5	548775	924330	8222567
6	548357	924462	8226070
Mean	548560	924437	8224842
% RSD	0.031	0.023	0.043

Table 3: Precision of the method for drospirenone, ethinyl estradiol and levomefolate.

Ethinyl estradiol			Levomefolate			Drospirenone		
Concentration (µg/mL)		Recovery (%)	Concentration (µg/mL)		Recovery (%)	Concentration (µg/mL)		Recovery (%)
Taken	Found		Taken	Found		Taken	Found	
1.2	1.196	99.66	18.04	17.953	99.52	120	119.484	99.57
1.2	1.196	99.66	18.04	17.943	99.46	120	119.556	99.63
1.2	1.196	99.63	18.04	17.950	99.50	120	119.568	99.64
1.2	1.195	99.61	18.04	17.953	99.52	120	119.436	99.53
1.2	1.196	99.68	18.04	17.948	99.49	120	119.472	99.56
1.2	1.195	99.60	18.04	17.950	99.50	120	119.520	99.60
Mean	1.196	99.64	**Mean**	17.949	99.50	**Mean**	119.506	99.59
RSD (%)	0.032	0.031	**RSD (%)**	0.022	0.023	**RSD (%)**	0.043	0.043

Table 4: Accuracy of the method for drospirenone, ethinyl estradiol and levomefolate.

Spiked level (%)	Concentration of drug (µg/mL)		Recovered (%)	RSD (%)
	Spiked	Found*		
Ethinyl estradiol				
50	0.60	0.599	99.803	0.067
100	1.20	1.196	99.66	0.067
150	1.80	1.792	99.57	0.038
Levomefolate				
50	8.93	8.893	100.61	0.109
100	17.86	17.949	100.50	0.010
150	26.79	26.903	100.56	0.119
Drospirenone				
50	60	59.767	99.62	0.075
100	120	119.477	99.56	0.041
150	180	179.873	99.93	0.061

Table 5: Recovery of drospirenone, ethinyl estradiol and levomefolate by the proposed method.

*Average of three determinations

Parameter Investigated	Ethinyl estradiol			Levomefolate			Drospirenone		
	Plate count	Peak Tailing	Resolution	Plate count	Peak Tailing	Resolution	Plate count	Peak Tailing	Resolution
Flow rate-0.9 mL/min	7966	1.38	-	8722	1.37	5.54	5564	1.70	2.89
Flow rate-1.1 mL/min	9778	1.39	-	10273	1.38	5.99	6344	1.79	3.00
Column temperature-25°C	8054	1.37	-	8658	1.38	5.58	5480	1.69	2.93
Column temperature-29°C	9653	1.39	-	10143	1.38	5.98	6426	1.77	3.00
Mobile phase ratio (0.1% H_3PO_4: methanol: acetonitrile) –60:25:15 *v/v/v*	8547	1.40	-	9155	1.42	5.75	5752	1.76	3.09
Mobile phase ratio (0.1 M NaH_2PO_4: methanol: acetonitrile) - 60:155:25 *v/v/v*	8588	1.39	-	9318	1.40	5.77	5827	1.74	3.03
Mobile phase pH–4.6	8501	1.40	-	9177	1.44	5.79	5849	1.76	3.20
Mobile phase pH–5.0	8516	1.41	-	9122	1.43	5.77	5799	1.75	3.19
Detection wavelength–243 nm	8496	1.41	-	8961	1.42	5.83	5715	1.77	3.24
Detection wavelength–247 nm	8412	1.39	-	9084	1.43	5.78	5789	1.73	3.24

Table 6: Robustness of the method.

of concentration 120 µg/mL of drospirenone, 1.2 µg/mL of ethinyl estradiol and 18.04 µg/mL of levomefolate. In each case, resolution, plate count and peak tailing were calculated. The calculated values were within the acceptance limits (Table 6). Therefore, the method is considered as robust.

Conclusion

For the first time, a stability indicating HPLC with photodiode array detector method has been developed and validated for the simultaneous assay of drospirenone, ethinyl estradiol and levomefolate in bulk and tablet dosage form. All validation parameters satisfied the acceptance criteria of the ICH guideline. The developed method is good enough to separate the peaks of drospirenone, ethinyl estradiol and levomefolate from the degradation products produced during stress degradation. Therefore, it was concluded the developed and validated stability indicating method can be employed for the routine estimation of drospirenone, ethinyl estradiol and levomefolate in quality control laboratories and for stability studies.

Acknowledgements

The author would like to thank Bharathiar University (Coimbatore, Tamil Nadu, India), Sacred Heart College (Autonomous) (Tirupattur, Tamil Nadu, India) and Rainbow Pharma Training Lab (Hyderabad, Telangana, India).

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