ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Stability Indicating HPLC Method for the Determination of Drospirenone in Pharmaceutical Dosage Forms.

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ABSTRACT

A stability indicating HPLC method has been proposed for the determination of drospirenone in pharmaceutical dosage forms. Analysis of drospirenone was carried out on a Nova-Pak CN column using a mixture of K_2HPO_4 50 mM and acetonitrile (60:40, v/v) adjusted to pH 8.0 as the mobile phase at a flow rate of 1 ml/min. The detection was performed at 245 nm at room temperature. The chromatographic method was linear over the range of 5-60 µg/ml with acceptable within-day and between-day coefficient of variations (CV<1.4%) and error values (<0.4%). Drospirenone was degraded under acidic, basic and oxidative conditions. On the other hand, drospirenone bulk powder was stable upon exposure to heat and light. The proposed method was found to be fast, simple, accurate, and reproducible for the determination of drospirenone in pharmaceutical dosage forms.

Keywords: Drospirenone, HPLC, Stability indicating, Stress degradation

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ISSN: 0975-8585

INTRODUCTION

Drospirenone (figure no. 1) is a synthetic progestin which is an analogue of spironolactone. Drospirenone also shows anti-mineralocorticoid and anti-androgenic properties [1]. Drospirenone in combination with ethinylestradiol is used as an oral contraceptive and also in the treatment of premenstrual dysphoric disorder, acne and hirsutism [1-3].

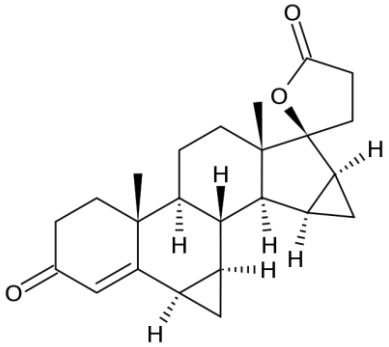

Figure 1: Chemical structure of drospirenone

An HPLC method is reported in the USP for the determination of drospinenone. Drug registration agencies require stability indicating analysis methods and usually pharmacopoeial methods do not fulfill these requirements. Few HPLC methods have been reported before for the determination of drospirenone in dosage forms [4-6]. In a recent paper the stress degradation of drospirenone has been studied using 0.1 M hydrochloric acid, 0.1 M sodium hydroxide, and 3% hydrogen peroxide for 30 min [7].

The present article describes comprehensive stress degradation on drospirenone by using various conditions and validating a stability indicating HPLC method for the determination of drospirenone in the presence of its degradation products. More intensive stress conditions were used in our laboratory to study the stress degradation of drospirenone.

EXPERIMENTAL

Chemicals

Drospirenone was from Shanghai Modern Pharmaceutical Co., Ltd., China (Batch No. 1106001) and kindly provided by Abouraihan Pharmaceutical Co., Tehran, Iran. Acetonitrile was of HPLC grade and purchased from Merck (Darmstadt, Germany). Purified water was prepared using a Milli-Q system (Millipore, USA). All other chemicals were of analytical grade and purchased from Merck.

Instrumentation

An HPLC system (Waters, Milford, MA, USA) equipped with a pump (Model 515), autosampler (Model 717 plus) and UV detector (Model 486) and a Chrom & Spec software (version 1.5 x) was used for chromatographic separation. The light sources include a 100 W Tungsten (visible light) and a low pressure Mercury lamp (UV light). Thermal studies were performed in a dry air oven (Melag dry air oven, Germany).

Chromatographic conditions

Chromatographic separation was carried out on a Nova-Pak CN column from Waters (Milford, MA, USA) by isocratic elution of a mixture of K_2HPO_4 50 mM and acetonitrile (60:40, v/v) adjusted to pH 8.0 as the mobile phase at a flow rate of 1 ml/min. The mobile phase was prepared daily and degassed before use. The HPLC analysis was performed at room temperature and the UV detector was set at 245 nm.

Yasmin tablets, containing 3.000 mg drospirenone and 0.030 mg ethinylestradiol, were from Bayer Schering Pharma AG (Germany) and purchased from a local pharmacy.

Standard solutions

Drospirenone stock standard solution (2500 µg/ml) was prepared by dissolving appropriate amount of the drug in acetonitrile.

System suitability solution of drospirenone was prepared by diluting the stock standard solution in the mobile phase to reach a concentration value of 25 µg/ml.

Linearity

Six series of drospirenone solutions for testing linearity were prepared by serial dilution of stock standard solution to sevendifferent concentration values of 5, 10, 20, 30, 40, 50, and 60 µg/ml in the mobile phase.

Accuracy and precision

The accuracy and precision of the method was evaluated by determination of drospirenone standard solutions in the mobile phase at three different concentration levels (5, 30, and 60 µg/ml). This experiment was performed in triplicate in one day and three consecutive days.

Robustness

To study the robustness of the proposed method, the effect of pH variation (±0.2) and also mobile phase composition (±2 ml) was studied on chromatographic parameters.

Stress degradation

An initial concentration of drodspirenone at 500 µg/ml was used for all stress degradation studies. Two ml of stock standard solution of drospirenone (2500 µg/ml) was transferred into a 10 ml volumetric flask. Then 1 M HCl, 0.1 M HCl, 0.1 M HCl, 1 M NaOH, and 0.01 M NaOH were added to each flask to reach the volume and the flasks were kept at room temperature or 80ºC to study the degradation of drospirenone. The resulted solutions were neutralized by appropriate amounts of NaOH or HCl and injected to the HPLC system after dilution to 25 µg/ml by mobile phase.

For oxidative degradation, 2 ml of stock standard solution of drospirenone and 8 ml of 3% or 1% hydrogen peroxide were transferred to a 10 ml volumetric flask and kept at room temperature or 80ºC.

For thermal and light degradation, a solid sample of drospirenone was spread in a thin layer in a watch glass and exposed to heat (80ºC) and light (visible and UV) for 5 days. Then, a standard solution was prepared at the concentration level of 25 µg/ml in the mobile phase and injected to the HPLC system.

A freshly prepared standard solution of drospirenone (25 µg/ml) was also injected to the HPLC system and the degradation of drospirenone in all degradation conditions was determined.

Application of proposed method

Twenty Yasmin tablets were weighed and crushed to a fine powder in a mortar and pestle. An amount of the powder equivalent to one tablet weighed accurately and transferred into a 100 ml volumetric flask. After addition of 70 ml of the mobile phase, the mixture was sonicated for 15 min. The flask was mode up to volume with mobile phase and a portion of the solition was centrifuged for 15 min at 4000 rpm and passed through a syringe filter (Teknokroma, Spain). The filtered solution was injected to the HPLC system. The peak area was compared with a standard solution at the same concentration value to find out the drug content.

Relative recovery

To assess the relative recovery, drospirenone samples prepared from the pharmaceutical formulation was spiked with standard solution of drospirenone. The resulted solution was treated according to the assay

ISSN: 0975-8585

method and injected to the HPLC system. The resulted peak area compared with a standard solution of drospirenone at the same concentration value to find out the relative recovery.

RESULTS AND DISCUSSION

Chromatographic conditions

The chromatographic separation was achieved on a Nova-Pak CN column using a mixture of K_2HPO_4 and acetonitrile (60:40, v/v) (pH 8.0) as the mobile phase. Under these conditions the drug substance showed a symmetrical peak with an acceptable retention time and no interference from the excipients or degradation products (figure no. 2).

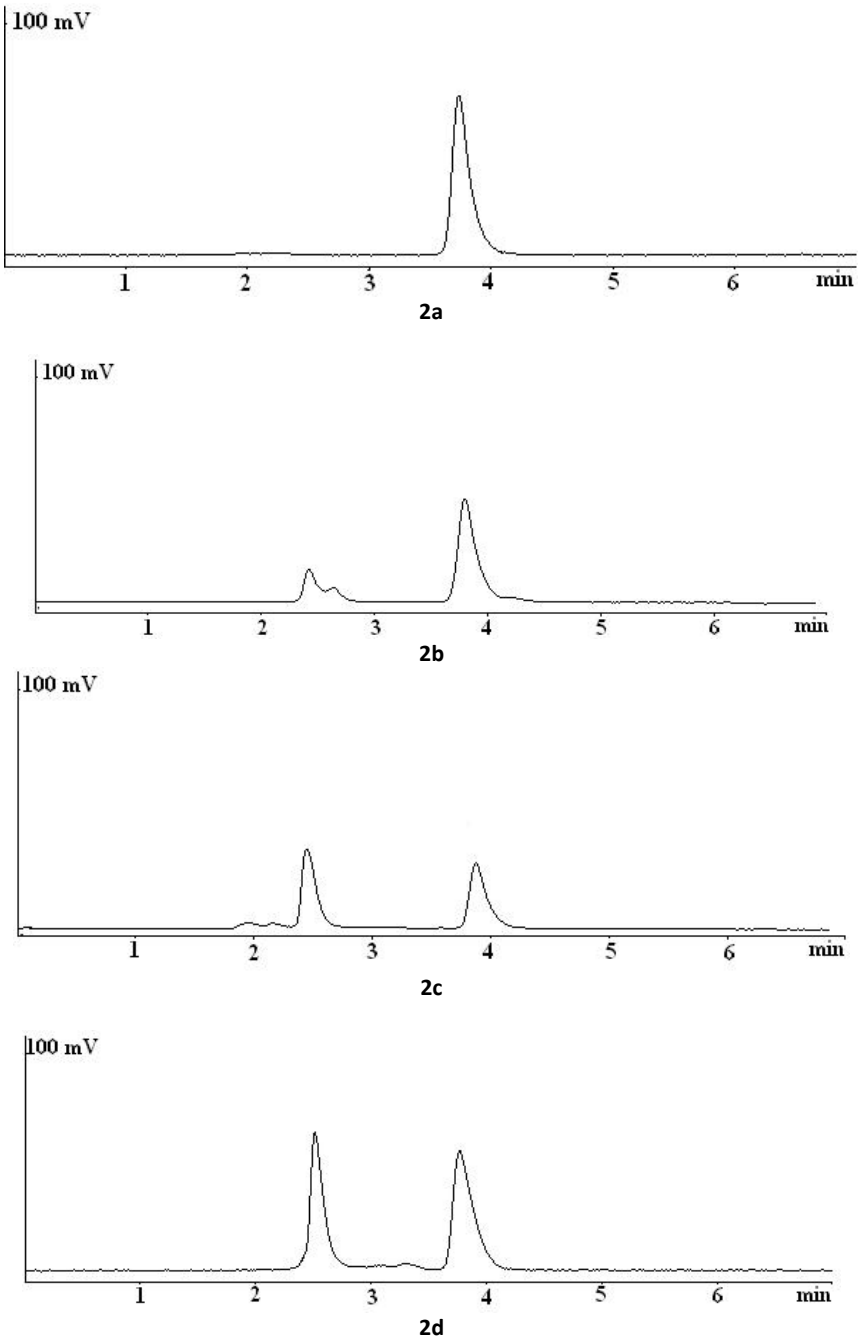

Figure 2: Typical chromatograms obtained from stability studies of drospirenone. (a) drospirenone standard solution (25 µg/ml); (b) drospirenone solution in 0.1 M HCl after 1 h at 80 °C; (c) drospirenone solution in 0.01 M NaOH after 15 min at room temperature; (d) drospirenone solution in 1% H_2O_2 after 1 h at 80 °C.

ISSN: 0975-8585

The system suitability parameters calculated from six replicate injections of drospirenone solution to the HPLC system are shown in Table no.1.

Table 1: System suitability parameters

Parameters	Found	Acceptable limits
USP theoretical plates (n = 6)	3100	N>1500
USP tailing factor (n = 6)	1.33	T<1.5
Repeatability (t_R) (n = 6)	0.48	RSD<1%
Repeatability (peak area)(n = 6)	0.96	RSD<1%

t_R: Retention time (min); N: Theoretical plate; T: Tailing factor; RSD: Relative Standard Deviation

Linearity

Six series of the calibration solutions in the range of 5-60 µg/ml were injected to the HPLC system and the calibration curves were constructed. The calculated statistical parameters are shown in Table no. 2. Excellent correlation was observed between the peak area and drospirenone concentration.

Table 2: Statistical data of calibration curves of drospirenone (n=6)

Parameters	Results
Linearity range	5-60 µg/ml
Regression equation	Y= 29.18X-4.47
Standard deviation of slope	0.19
Relative standard deviation of slope (%)	0.65
Standard deviation of intercept	2.99
Correlation coefficient (r^2)	0.9997
Limit of quantification (LOQ)	1.02 µg/ml
Limit of detection (LOD)	0.34 µg/ml

Accuracy and precision

The data obtained from accuracy and precision experiments are shown in Table no. 3. The CV values for the within-day and between-day were less than 1.4% which confirms sufficient reproducibility of the proposed method.

Table 3: Precision and accuracy of the method for determination of drospirenone (3 sets for 3 days)

Concentration added (µg/ml)	Concentration found (µg/ml)	CV (%)	Error (%)
Within day (n = 3)			
5.00	5.02±0.06	1.20	0.40
30.00	29.96±0.42	1.40	-0.13
60.00	59.95±0.22	0.37	-0.08
Between day (n = 9)			
5.00	5.01±0.06	1.20	0.20
30.00	29.93±0.34	1.14	-0.23
60.00	60.15±0.50	0.83	0.25

To establish the ruggedness of the proposed method, a standard solution of drospirenone was quantified by two different analysts on two different HPLC systems. The results showed insignificant variations (CV<2%).

Robustness

The results for the robustness of the method showed that the overall RSD values for the peak areas were less than 2% which is acceptable. The retention time of drospirenone was changed by different organic phase composition, but there was no problem with peak shape or separation of drospirenone from degradation peaks.

Analysis of pharmaceutical product

The proposed HPLC method was applied for the determination of drospirenone in Yasmin tablets. Drospirenone amount was shown to be 3.08±0.01 mg which is in agreement with the labeled amount (3.00 mg).

Recovery

The percentage recovery was calculated by standard addition method. The obtained recovery was 100.4±0.5 and no interferences were observed due to excipients at the retention time of drospirenone.

Degradation studies

The results of the stress degradation of derospirenone in different conditions are shown in Table no. 4. Drospirenone degraded slowly in 0.1 M hydrochloric acid. The peak area of drospirenone was decreased about 34% after 1 h exposure at80ºC. Two new peaks were generated between the retention times of 2 and 3 min (figure no. 2b).

Table 4: The results of the stress degradation tests on drospirenone bulk powder using different conditions

Stress test condition	Solvent	Temperature	Time	% of drospirenone
Acidic	1 M HCl	Room temperature	30 min	81.1
	1 M HCl	80ºC	30 min	24.5
	0.1 M HCl	80ºC	1 h	66.4
Basic	1 M NaOH	Room temperature	30 min	20.4
	0.01 M NaOH	Room temperature	15 min	44.1
Oxidative	3% H_2O_2	Room temperature	30 min	93.8
	3% H_2O_2	80ºC	30 min	18.9
	1% H_2O_2	80ºC	1 h	81.1
Photolytic				
UV light	Solid form	Room temperature	5 days	100.3
Visible light	Solid form	Room temperature	5 days	99.9
Heat	Solid form	80 °C	5 days	100.1

In basic conditions, drospirenone was found to be more labile. The drug was degraded about 80% after 30 min exposure to 1 M NaOH at room temperature. The degradation was slower under exposure to 0.01 M NaOH and 56% degradation was observed after 15 min at room temperature. A new peak at the retention time of about 2.4 was appeared in the chromatogram (figure no. 2c).

Drospirone was found to degrade in 1% H_2O_2 to an extent of 19% after 1h. More degradation was observed by using 3% H_2O_2 at room temoerature or 80ºC (Table no. 4 and figure no. 2d).
Drospirenone bulk powder was stable under exposure to heat, UV light, and visible light and no significant degradation was observed (Table no. 4).

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