

**COMPREHENSIVE STUDIES OF ANALYTICAL METHODS FOR DROSPIRENONE  
AND ETHINYL ESTRADIOL**

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**ABSTRACT**

Oral contraceptive (OC) pills contain Estrogen and progestin that are synthetic analogs of natural hormones. These synthetic hormones affect the hypothalamus-pituitary-gonadal axis of the female reproductive system. There are many types of contraceptives; most of the OC pills prevent pregnancy by inhibiting ovulation. Drospirenone is a novel progestin with anti-mineralocorticoid and anti-androgenic properties, while ethinylestradiol is a synthetic estragon. The synergistic effects of these two hormones make this combination an effective and versatile option for various women's health applications. This article aims to provide comprehensive details regarding drospirenone and ethinylestradiol, including information on many contraceptive pills along with its concentration, mechanism of action, side effects and various analytical techniques such as RP HPLC, HPLC, UV, LC and HPTLC. The article will also elucidate different analytical methods used in recent studies.

**KEYWORDS:** Drospirenone, Ethinyl estradiol, HPLC, UV, RP-HPLC and HPTLC.

**INTRODUCTION**

Oral contraceptive is best optional method to prevent pregnancy due to modernized lifestyle. Oral contraceptives are used not only to prevent pregnancy but they also reduce the risk of endometrial and ovarian cancer and protect against acute pelvic inflammatory disease and ectopic pregnancies.<sup>[1]</sup>

called fertilization. The hormones in the pill safely stop ovulation. No ovulation means there's no egg for sperm to fertilize, so pregnancy can't happen. The pill's hormones also thicken the mucus on the cervix. This thicker cervical mucus blocks sperm so it can't swim to an egg — kind of like a sticky security guard.<sup>[2]</sup>

The birth control pill works by stopping sperm from joining with an egg. When sperm joins with an egg it's

**Table 1: List of Synthetic Hormonal Contraceptive Pills Available, Their Mode of Action and Their Side Effect.**

Name	Active component	Mode of action	Side effects	Ref.
Estradiol valerate	Estradiol valerate 2 mg	Estrogen diffuses into their target cells (i.e., cells in the female reproductive tract, mammary glands, hypothalamus, and pituitary) and bind to receptor proteins	Abnormal hair growth, Breast tenderness, changes in sex drive, cramps, dizziness, hair loss, headache, light headedness	[3]
Femilon	DSG BP 0.15 mg Ethinyl estradiol IP 0.02 mg	Once bound to the receptor, progestins like DSG will slow the frequency of GnRH from the hypothalamus and blunt the pre-ovulatory LH surge. Femilon contraceptive pill unleashes ethinyl estradiol and DSG into the blood stream	Vaginal infections, urinary tract infections, Breast pains and engorgement, auditory disturbances	[4]
CPA and ethinyl estradiol	CPA 2 mg ethinylestradiol 0.035	Binds to the progesterone and Estrogen receptors slows the release of GnRH	Blood clots, cancers such as breast or cervical cancer	[5]

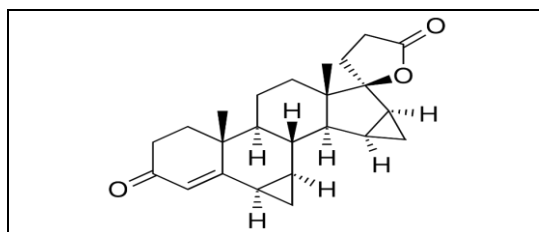
	mg	from the hypothalamus and blunt the pre-ovulatory LH surge		
Estrogen and progestin	GSD BP 60 mcg ethinyl estradiol 15 mcg	Estrogen increase the hepatic synthesis of SHBG and other serum proteins and suppress FSH from the anterior pituitary. The combination of an Estrogen with a progestin suppresses the hypothalamic-pituitary system, decreasing the secretion of GnRH	Severe chest pain and cough of acute onset, severe headache, vision problems, dizziness	[6]
DSG and ethinylestradiol tablets	DSG 0.15 mg ethinylestradiol 0.03 mg	Binds to the Estrogen and progesterone receptor, inhibits ovulation	Severe allergic reactions, bloody diarrhoea, breast lumps pain or discharge fainting, frequent or painful urination migraines, missed menstrual period	[7]
Ovipauz levonorgestrel	Levonorgestrel IP 0.15 mg ethinylestradiol 0.03 mg	It inhibits ovulation, prevents transport of sperm or eggs and thus prevents fertilization and alters the lining of the uterus to prevent pregnancy	Ovipauz-levonorgestrel may cause thrombotic and thromboembolic disorders, vascular problems, hepatic neoplasia, carcinoma of breasts and reproductive organs, gallbladder disease, ocular lesions	[8]
Crisanta LS	Ethinyl estradiol 0.02 mg DRSP 3 mg	Progestins such as DRSP diffuse freely into target cells in the female reproductive tract and bind to the progesterone receptor. And block the GnRH release and LH surge	Darkening of facial skin, allergy, mood swings	[9]
Duoluton levonorgestrel	Levonorgestrel IP 0.25 mg ethinylestradiol 0.05 mg	Levonorgestrel tricks the body processes into thinking that ovulation has already occurred, by maintaining high levels of the synthetic progesterone. This prevents the release of eggs from the ovaries	Local skin reaction, depression, liver impairment, reduce menstrual loss	[10]

DRSP: Drospirenone, CPA: Cyproterone acetate, DSG: Desogestrel, GSD: Gestodene, SHBG: Sex hormone binding globulin, GnRH: Gonadotropin-releasing hormone

### PHYSICAL AND CHEMICAL PROPERTIES OF DRUGS

Drospirenone is a progestin and antiandrogen medication which is used in birth control pills to prevent pregnancy and in menopausal hormone therapy, among other uses.<sup>[11]</sup> The compound is a norpregnane derivative, with the IUPAC name Norpregn-4-en-20-yn-3,17-diol. It is an estragon, exhibiting a molar mass of 296.410 g·mol<sup>-1</sup> and a melting point range of 182 to 184 °C. Its

bioavailability ranges from 38% to 48%, and it is soluble in various organic solvents and vegetable oils. In aqueous solutions, it has a solubility of 4.83 mg/L. The drug acts by suppressing ovulation, inhibiting gonadotrophic hormone, thickening cervical mucus to impede sperm travel, and preventing changes in the endometrium necessary for fertilized egg implantation.



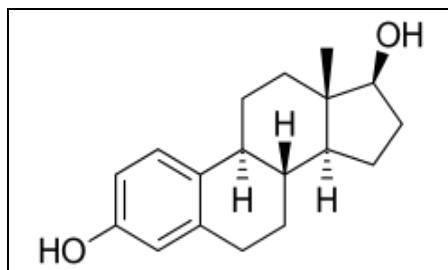
Structure of drospirenone

Ethinyl estradiol Ethinyl estradiol is a synthetic derivative of the naturally occurring female sex hormone, estradiol. Ethinyl estradiol can be used in

hormone replacement therapy to alleviate symptoms associated with menopause, such as hot flashes, vaginal dryness, and mood swings. The compound, with the

IUPAC name 19-Nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,17-diol, has a chemical formula of C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>, a molar mass of 296.410 g·mol<sup>-1</sup>, and a melting point ranging from 182 to 184 °C. It falls under the drug category of Estrogen and exhibits a bioavailability of 38–48%. This substance demonstrates solubility in various solvents such as Ethanol, Ether, Acetone, Dioxane, Chloroform, vegetable oils, and solutions of fixed alkali hydroxide

(NaOH, KOH). In double-distilled water, its solubility is measured at 4.83 mg/L. The mechanism of action involves suppressing ovulation by inhibiting gonadotrophic hormone, thickening cervical mucus to impede sperm travel, and preventing the necessary endometrial changes for the implantation of a fertilized egg.<sup>[12]</sup>



Structure of ethinyl estradiol

**Table 2: Analytical Method For Ethinyl Estradiol And Drospirenone In Bulk And Pharmaceutical Dosage Forms.**

S. NO	TITLE	METHOD DESCRIPTION	REF. NO	YEAR OF PUBLISHED
1	RP-HPLC method to assay ethinylestradiol and drospirenone with 3D chromatograph model in fixed dose in rat plasma for pharmacokinetic study analysis	<b>Fluorescence detection:</b> (EE) $\lambda=$ 200-310 nm <b>Ultraviolet-visible (UV/Vis) detection:</b> (DP) 270 nm. <b>Retention Times:</b> EE 4.19 and DP 5.30 minutes. <b>(LOD) and (LOQ):</b> EE 0.121 and 0.282 $\mu$ g/mL <b>LOD and LOQ:</b> DP 2.23 and 7.697 $\mu$ g/mL <b>(r<sup>2</sup>) value:</b> EE 0.9937 and DP 0.9913 <b>RSD:</b> less than 5%.	13	July 2023
2	Simultaneous determination of ethinyl estradiol and drospirenone in oral contraceptive by high performance liquid chromatography	<b>Column:</b> LiChroCART® 100RP column (125x4 mm i.d., 5 $\mu$ m) <b>Mobile phase:</b> acetonitrile: water 50:50 (v/v) <b>Flow rate:</b> 1.0 mL.min <sup>-1</sup> <b>Fluorescence detection:</b> EE $\lambda$ max= 280 nm and $\lambda$ max= 310 nm <b>UV detection:</b> DP 200 nm <b>Elution time:</b> EE 4.0 and DP 5.7 min.	14	September 2013
3	RP-HPLC Method Development and Validation for Estimation of Drospirenone and Ethinyl Estradiol in Bulk and Combined Dosage Form	<b>Column:</b> Phenomenex Luna C18 (250mm x 4.6mm, 5 $\mu$ m particle size) <b>Column temperature:</b> ambient temperature <b>Mobile phase:</b> acetonitrile: water (60:40, v/v) <b>Flow rate:</b> 1.5 ml/min <b>UV detection:</b> 280 nm <b>Retention time:</b> DP 2.7 and EE 5.3 minutes. <b>Linearity:</b> 80-400 $\mu$ g/ml and 1.4-7.0 $\mu$ g/ml <b>Correlation coefficients:</b> 0.997 DP and EE 0.996	15	2013
4	Quantification of Drospirenone- and Ethinyl Estradiol-Related Impurities in a Combined Pharmaceutical Dosage Form by a Chromatography Method with a QbD Robustness Study	<b>Column:</b> Agilent Zorbax SB C18 column (4.6 mm x 250 mm, 5 $\mu$ m) <b>Wavelength:</b> 215 nm <b>Mobile phases:</b> (A) (100% acetonitrile) and (B) (acetonitrile-water, 1 + 3, v/v) <b>Flow rate:</b> 1.3 mL/min <b>Column temperature:</b> 40°C. <b>Linearity:</b> 1.5 to 90 $\mu$ g/mL for DP and 0.125 to 0.75 $\mu$ g/mL for EE.	16	January 2024
5	Development and validation of a simple and sensitive rp-hplc method for simultaneous estimation of	<b>Column:</b> a column of WATERS C18 (250 x 4.6 mm, 5 $\mu$ m.) <b>Pump:</b> 1525 Binary HPLC pump	17	OCTOBER 2012

	drospirenone and ethinylestradiol in combined tablet dosage form	<b>UV Spectrophotometer:</b> SHIMADZU UV180, <b>Flow rate:</b> 1.0ml/min <b>Wavelength:</b> 275 nm <b>Mobile phase:</b> Acetonitrile and 10mM Formic Acid (70:30) <b>Retention times:</b> DP 4.15 min and EE 2.25 min. <b>Linearity range:</b> 50µg/ml to 150µg/ml		
6	Simultaneous determination of ethinyl estradiol and drospirenone in oral contraceptive by high performance liquid chromatography	<b>column:</b> LiChroCART® 100RP column (125x4 mm i.d., 5 µm) <b>Mobile phase:</b> acetonitrile: water 50:50 (v/v) <b>Flow rate:</b> 1.0 mL.min <sup>-1</sup> <b>Fluorescence detection:</b> EE λ max= 280 nm and λ max= 310 nm <b>UV detection:</b> DP 200 nm. <b>Elution time:</b> EE 4.0 and DP 5.7 min,	18	SEPTEMBER 2013
7	Method Development and Validation for Simultaneous Estimation of Ethinyl Estradiol and Drospirenone and Forced Degradation Behavior by HPLC in Combined Dosage Form	<b>Column:</b> Thermo Hypersil BDS C18 Column (4.6×250 mm and 5 µm) <b>Flow rate:</b> 1.0 ml/min <b>Buffer:</b> Acetonitrile and ammonium acetate buffer <b>Wavelength:</b> 258 nm <b>Linearity:</b> EE 0.06- 0.18 µg/ml, and DP 6-18 µg/ml <b>Retention Time:</b> EE 1.4 min and DP 5.3 min	19	APRIL 2013
8	Validated HPTLC method for simultaneous estimation of ethinyl estradiol and drospirenone in bulk drug and formulation	<b>Stationary phase:</b> silica gel 60 F 254 <b>Solvent:</b> toluene/methanol/ammonia (8:2:0.1) (v/v/v). <b>Wavelength:</b> 280 nm. <b>R<sub>f</sub> values:</b> ethinyl estradiol 0.29 ± 0.02 and drospirenone 0.42 ± 0.02. <b>Linearity:</b> (150 – 400 ng/spot for ethinyl estradiol and 30 – 80 ng/spot for drospirenone)	20	FEBRUARY 2012
9	Ultrasound-Assisted Extraction, Followed by Gas Chromatography–Mass Spectrometry for the Simultaneous Quantification of Ethinyl Estradiol and Drospirenone in Contraceptive Formulations	<b>Method:</b> GC-MS <b>Extraction:</b> extracted from the solid by ultrasound-assisted extraction (15 min) in methanol. <b>Calibration range:</b> EE (3–12 µg mL <sup>-1</sup> ) and DP (300–1200 µg mL <sup>-1</sup> ) <b>R<sup>2</sup> values:</b> exceeding 0.99. <b>Recovery rates:</b> 106 ± 8% for EE and 93 ± 9% for DP <b>Standard deviation values:</b> below 6% for both analytes. <b>Limits of detection:</b> 0.25 µg mL <sup>-1</sup> for EE and 6.6 µg mL <sup>-1</sup> for DP. <b>Limits of quantification:</b> 0.82 µg mL <sup>-1</sup> for EE and 22 µg mL <sup>-1</sup> for DP	21	JUNE 2023
10	Development and Validation of New Analytical Method for Simultaneous Estimation of Drospirenone and Ethinyl Estradiol	<b>Solvent:</b> methyl alcohol <b>Absorbance:</b> Drospirenone has absorbance maxima 242nm and ethinyl estradiol has absorbance maxima 218nm <b>Concentration range:</b> 10-50µg/ml drospirenone and 32-38µg/ml for ethinyl estradiol.	22	2019
11	A Rapid Derivative Spectrophotometric Method for Simultaneous Determination of Ethinylestradiol and Drospirenone in Dosage Forms	<b>Wavelength:</b> EE 211 nm and DP 298 and 302 nm <b>Concentration range:</b> EE 0.25-2.5 µg/mL and DP 20-200 µg/ml.	23	JUNE 2018
12	Analytical method development & validation for simultaneous estimation of drospirenone & ethinyl estradiol in its dosage form	<b>Column:</b> C-18 (250 mm × 4.6 mm, 5.0 µ) <b>Mobile phase:</b> Acetonitrile:Water (60:40 v/v) <b>Flow rate:</b> 1 ml/min <b>Wavelength:</b> 225 nm <b>Retention time:</b> Ethinylestradiol 5.69 and Drospirenone 6.79 min <b>Concentration range:</b> 5-15 µg/ml for Ethinylestradiol	24	JUNE 2013

		and 50-150 µg/ml for Drospirenone. <b>LOD and LOQ:</b> 0.585 µg/ml and 1.772µg/ml for Drospirenone and 0.057 µg/ml and 0.174 µg/ml for Ethinylestradiol. <b>% RSD:</b> below 2.0		
13	Efficient, Cost-Effective Analytical Method Validation for Simultaneous Estimation of Related Substances in Ethinyl Estradiol & Drospirenone by HPLC in Combined Dosage Form	<b>Colum:</b> Oyster BDS premium (150mmX4.6mm, 3µm) <b>Injection volume:</b> 10MI <b>Colum temperature:</b> 30°C <b>Detection:</b> 245 nm for drospirenone &210 nm for Ethinyl estradiol <b>Retention Time:</b> Ethinyl estradiol 39 minutes and drospirenone 46 minutes	25	July 2020
14	Determination of ethinylestradiol and drospirenone in oral contraceptives with HPLC method with UV and fluorescence detection	<b>Colum:</b> Purospher STAR RP-18e reversed-phase column (150 x 4.0 mm I.D.; 5 µm) <b>Mobile phase:</b> 47% acetonitrile: 53% water (V/V). <b>Flow rate:</b> 1.50 ml /min <b>Temperature:</b> room temperature (24 ± 2°C). <b>UV absorbance:</b> DP at 265 nm and EE at 310 nm (excitation at 285 nm) <b>Concentration range:</b> EE 0.6 to 3.0 µg/ml and DP 60.0 to 300.0 µg/ml	26	2009
15	A Validated LC Method for the Simultaneous Determination of Drospirenone and Ethinylestradiol in Tablet Dosage form by Using Combine Fluorescence and UV Detectors	<b>Colum:</b> XTerra Phenyl 150 x 4.6 mm 5µ <b>Mobile phase:</b> water and acetonitrile in the ratio of 35:65 v/v <b>Flow rate:</b> 1.0 ml/min <b>Wavelength:</b> 260 nm DP and 285 nm Excitation, 310 nm Emission EE. <b>LOD and LOQ:</b> DP 0.02 and 0.05ug/mL and EE 0.21 and 0.45 ng/mL	27	JANUARY 2013
16	Analytical Method Development and Validation for Simultaneous Estimation of Ethinyl Estradiol and Drospirenone by HPLC in Combined Dosage Form	<b>Colum:</b> ProntoSil C18 ace-EPS,30cmX3.0 mm, followed in series by chromalith RP-18E C18, 10cm x 4.6 mm, 3µm <b>Colum Temperature:</b> 40°C <b>Sample Temperature:</b> 25°C <b>Injection Volume:</b> 20µL <b>Run time:</b> 70 minutes <b>Retention time of</b> a) Drospirenone: 34 min b) Ethinyl Estradiol: 47 min	28	JULY 2014

## CONCLUSION

This article has provided a comprehensive exploration and elucidation of the advancements in the combined analysis of Drospirenone and ethinyl estradiol up to the present day. The review encompasses an in-depth examination of various analytical methods employed for the simultaneous determination of Drospirenone and ethinyl estradiol, shedding light on the diverse approaches and techniques applied in both bulk and pharmaceutical dosage forms. This comprehensive study delves into a detailed review and analysis of various analytical methods encompassing UV, HPLC, RP-HPLC, LC, HPTLC, specifically focusing on their application in the analysis of Drospirenone and ethinyl estradiol. The research incorporates exhaustive details on their concentrations, mechanisms of action, and potential side effects, offering a holistic understanding for those considering or currently using these contraceptives and providing a comprehensive understanding of the

analytical methodologies employed in pharmaceutical analysis.

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