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Review Article

A Review: Method Development and Validation for Simultaneous Estimation of Drospirenone and Estetrol

Sayad Althaf[1],Telang Priyanka[2]*, Namrata Shivankar[3]

Department of pharmaceutical chemistry, Channabasweshwar Pharmacy College (degree) Latur, Maharashtra, India.

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ABSTRACT

The simultaneous estimation of combined oral contraceptive drugs such as drospirenone and estetrol has gained significant importance in pharmaceutical analysis due to their widespread clinical use and regulatory requirements for quality control. This review focuses on the development and validation of advanced chromatographic techniques, particularly reverse phase high-performance liquid chromatography (RP-HPLC) and its modern counterpart, ultra-performance liquid chromatography (UPLC), for the quantitative determination of these drugs in bulk and pharmaceutical dosage forms. Various analytical methods reported in the literature demonstrate the use of C18 columns with mobile phases consisting of combinations of buffers such as orthophosphoric acid or phosphate buffer and organic solvents like acetonitrile or methanol, under isocratic conditions. The reviewed methods exhibit excellent system suitability with retention times generally below 3 minutes, ensuring rapid analysis. Validation parameters including linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ) were found to comply with ICH guidelines. The linearity of both drugs was consistently observed with correlation coefficients (r^2) close to 0.999, indicating high sensitivity and reliability. Accuracy studies showed recovery values within 98–102%, while precision studies reported %RSD values less than 2%, confirming the reproducibility of the methods. Additionally, stability-indicating methods demonstrated effective separation of degradation products under stress conditions such as acid, base, oxidative, thermal, and photolytic degradation. Recent advancements highlight the transition from conventional HPLC to UPLC techniques, offering advantages such as reduced run time, improved resolution, enhanced sensitivity, and lower solvent consumption. These improvements make UPLC a more efficient and eco-friendly alternative for routine pharmaceutical analysis.

***Corresponding Author:** Telang Priyanka
Address: *Department of pharmaceutical chemistry, Channabasweshwar Pharmacy College (degree) Latur, Maharashtra, India*
Email ✉: priyankatelang99@gmail.com
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Overall, the compiled studies confirm that RP-HPLC and UPLC methods are simple, accurate, precise, cost-effective, , and suitable for routine quality control and stability testing of drospirenone and estetrol in combined dosage forms.

INTRODUCTION

Analytical chemistry plays a crucial role in the pharmaceutical field by ensuring the quality, safety, and efficacy of drug substances and dosage forms. Among various analytical techniques, chromatographic methods, particularly reverse-phase high-performance liquid chromatography (RP-HPLC) are widely employed for the separation, identification, and quantification of pharmaceutical compounds due to their high sensitivity, accuracy, and reproducibility. In recent years, advancements such as ultra-performance liquid chromatography (UPLC) have further enhanced analytical efficiency by reducing analysis time, improving resolution, and minimizing solvent consumption [1–4,10–15].

Drospirenone is a synthetic progestin widely used in oral contraceptive formulations for the prevention of pregnancy. It exhibits anti-mineralocorticoid and anti-androgenic properties, contributing to its therapeutic effectiveness in managing conditions such as acne, premenstrual dysphoric disorder (PMDD), and hormonal imbalance. It functions by inhibiting ovulation through suppression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), along with altering cervical mucus and endometrial conditions to prevent fertilization and implantation [1–6].

Estetrol, on the other hand, is a naturally occurring estrogen produced during pregnancy and is now synthesized for pharmaceutical use. It exhibits selective activity on estrogen receptors (ER-α and ER-β), offering improved safety and reduced environmental impact compared to traditional synthetic estrogens such as ethinyl estradiol. Due to its tissue-selective action, estetrol demonstrates estrogenic effects in reproductive tissues while minimizing adverse effects on breast tissue [6–8].

The combination of drospirenone and estetrol in a fixed-dose formulation represents a significant advancement in oral contraceptive therapy, providing effective pregnancy prevention with improved safety profiles. However, the simultaneous estimation of these drugs in bulk and pharmaceutical dosage forms presents analytical challenges due to their structural differences and varying physicochemical properties [12].

A review of the literature reveals that several analytical methods have been developed for the determination of drospirenone and estetrol, either individually or in combination, using techniques such as RP-HPLC, UPLC, and UV spectrophotometry. Among these, RP-HPLC remains the most preferred method due to its robustness, specificity, and suitability for routine quality control analysis. These methods typically employ C18 columns with mobile phases consisting of buffers and organic solvents under isocratic conditions, ensuring efficient separation with short retention times [14].

Furthermore, method validation in accordance with International Council for Harmonisation (ICH) guidelines is essential to ensure the reliability and reproducibility of analytical results. Key validation parameters include linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ), all of which are critical for establishing method suitability in pharmaceutical analysis [31–35].

This review aims to comprehensively summarize and critically evaluate the various RP-HPLC and emerging UPLC methods reported for the

simultaneous estimation of drospirenone and estetrol. Emphasis is placed on method development strategies, validation parameters, and recent advancements to provide insights into efficient analytical approaches for routine quality control and stability studies [36–50].

Syed Ismail Jabiullah et al. (2022):

A study by Syed Ismail Jabiullah et al. (2022) reported the development and validation of a simple, precise, and accurate RP-HPLC method for the simultaneous estimation of drospirenone and estetrol in bulk and pharmaceutical dosage forms.

The chromatographic separation was achieved using an Agilent HPLC system equipped with a PDA detector and a C18 column (150 mm × 4.6 mm, 3.5 µm). The mobile phase consisted of orthophosphoric acid and acetonitrile in the ratio of 55:45, operated in isocratic mode. The flow rate was maintained at 1.0 mL/min, with a detection wavelength of 240 nm, column temperature at 30°C, and injection volume of 10 µL.

The method demonstrated efficient separation with retention times of 3.011 minutes for drospirenone and 2.403 minutes for estetrol, indicating rapid analysis and reduced runtime. System suitability parameters such as plate count (>2000), tailing factor (<2), and resolution (>2) were within acceptable limits, confirming good chromatographic performance (observed in Table 1, page 4).

Linearity was established over concentration ranges of 1.5–9 µg/mL for drospirenone and 7.1–42.6 µg/mL for estetrol, with correlation coefficients (r^2) of 0.999, demonstrating excellent linear relationships (Table 3, page 5). The regression equations obtained were y = 11789x + 0.285 for drospirenone and y = 96131x + 8828 for estetrol, indicating high sensitivity of the method.

Accuracy studies performed using the standard addition method showed mean recoveries of 99.51% for drospirenone and 100.29% for estetrol, which fall within acceptable limits (98–102%), confirming the accuracy of the method (Tables 4 and 5, pages 6–7).

Precision studies indicated %RSD values of 0.3% for drospirenone and 0.8% for estetrol, demonstrating excellent repeatability and reproducibility (Table 6, page 7). Robustness testing under slight variations in flow rate, mobile phase composition, and temperature showed %RSD values within limits, confirming the reliability of the method (Table 7, page 7–8).

The method also exhibited good sensitivity with LOD values of 0.09 µg/mL for drospirenone and 0.36 µg/mL for estetrol, and LOQ values of 0.26 µg/mL and 1.10 µg/mL, respectively (Table 9, page 8).

Assay results of marketed formulations showed 99.87% for drospirenone and 100.07% for estetrol, confirming the applicability of the method for routine quality control analysis (Table 8, page 8).

Overall, the developed RP-HPLC method was found to be simple, rapid, accurate, precise, robust, and economical, making it suitable for routine pharmaceutical analysis and quality control of combined dosage forms containing drospirenone and estetrol. [1]

Geetha Rani Rachapudi et al. (2024)

A study by Geetha Rani Rachapudi et al. (2024) developed a stability-indicating RP-HPLC method for the simultaneous estimation of drospirenone and estetrol in combined pharmaceutical dosage forms. The method also evaluated the forced

degradation behavior of both drugs under various stress conditions, highlighting its applicability for stability studies.

Chromatographic separation was achieved using a Waters SunFire C18 column with a mobile phase consisting of 0.01N ammonium phosphate buffer and acetonitrile (70:30 v/v). The analysis was performed at a flow rate of 1.0 mL/min, with an injection volume of 20 µL, and detection was carried out at a wavelength of 265 nm using a UV detector.

The method exhibited efficient separation with retention times of approximately 2.8 minutes for drospirenone and 2.2 minutes for estetrol, indicating a rapid analytical process. System suitability parameters such as tailing factor (~1.2) and theoretical plate count (7860 for drospirenone and 6459 for estetrol) confirmed good chromatographic performance (Table 1, page 3).

Linearity was observed in the concentration range of 1.5–9 µg/mL for drospirenone and 14.2–85.2 µg/mL for estetrol, with excellent calibration curves (Table 2, page 5). Accuracy studies showed mean recoveries of 100.15% for drospirenone and 100.39% for estetrol, demonstrating the reliability of the method (Table 3, page 5).

Precision studies indicated low %RSD values of 0.3–0.5% for drospirenone and 0.4–0.7% for estetrol, confirming the reproducibility of the method (Tables 4 and 5, page 6). Robustness testing under small variations in analytical conditions (flow rate and temperature) showed no significant effect on results, indicating method robustness.

The sensitivity of the method was confirmed with LOD values of 0.04 µg/mL (drospirenone) and 0.13 µg/mL (estetrol), and LOQ values of 0.13 µg/mL and 0.40 µg/mL, respectively (page 7).

A key highlight of this study is the forced degradation analysis, where both drugs were subjected to stress conditions such as acid, base, oxidative, thermal, UV, and hydrolytic degradation. The results demonstrated that the method could effectively separate degradation products, confirming its stability-indicating capability (Table 6, page 7).

The assay of marketed tablet formulation showed 100.2% for drospirenone and 99.42% for estetrol, indicating good agreement with label claims. Overall, the developed RP-HPLC method was found to be simple, precise, accurate, rapid, and suitable for routine quality control as well as stability studies. [7]

L. Swathi et al. (2023)

A validated stability-indicating RP-HPLC method for the simultaneous determination of drospirenone and estetrol was developed by L. Swathi et al. (2023). The method employed an Agilent C18 column (150 × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1N KH_2PO_4 buffer and acetonitrile in the ratio of 50:50, delivered at a flow rate of 1.0 mL/min. The detection was carried out at 263 nm, and the column temperature was maintained at 30°C. The developed method showed efficient chromatographic separation with retention times of 2.268 minutes for estetrol and 2.698 minutes for drospirenone, indicating a rapid and efficient analytical procedure. System suitability parameters were within acceptable limits, with %RSD values of 0.5 for estetrol and 0.8 for drospirenone, confirming the reliability of the system.

The method exhibited good linearity over the concentration ranges of 6–36 µg/mL for estetrol and 0.75–4.5 µg/mL for drospirenone, with regression equations of y = 26009x + 3120.7 and y = 25028x + 719.84, respectively, indicating

strong correlation and sensitivity. Accuracy studies carried out using the standard addition method showed mean recoveries of 100.91% for estetrol and 99.76% for drospirenone, demonstrating the accuracy of the method. Precision studies, including system precision and method precision, showed %RSD values less than 2%, confirming excellent repeatability and reproducibility of the analytical method.

Robustness studies performed by making small deliberate changes in chromatographic conditions such as flow rate, mobile phase composition, and temperature showed no significant variation in results, indicating that the method is robust. The sensitivity of the method was confirmed with LOD and LOQ values of 0.39 µg/mL and 1.18 µg/mL for estetrol, and 0.01 µg/mL and 0.03 µg/mL for drospirenone, respectively.

Furthermore, forced degradation studies were conducted under various stress conditions including acidic, basic, oxidative, thermal, photolytic, and hydrolytic environments. The results revealed that both drugs showed maximum degradation under acidic and oxidative conditions, while minimal degradation was observed under thermal, UV, and hydrolytic conditions. Importantly, no interference from degradation products was observed at the retention times of the analytes, confirming the stability-indicating nature of the method.

The developed method was successfully applied to the analysis of marketed tablet formulations (DESOGEN), where the percentage assay was found to be 99.64% for estetrol and 99.89% for drospirenone, which is within acceptable limits. Overall, the method was found to be simple, accurate, precise, robust, and suitable for routine quality control as well as stability testing of drospirenone and estetrol in combined dosage forms. [4]

Rafi Syed and Rambabu Kantipudi (2021)

A novel and validated reverse phase ultra-performance liquid chromatography (RP-UPLC) method for the simultaneous estimation of drospirenone and estetrol in active pharmaceutical ingredients and tablet dosage forms was developed by Rafi Syed and Rambabu Kantipudi (2021). The chromatographic separation was achieved using a Luna C18 column (100 × 2.6 mm, 1.6 µm) with a mobile phase consisting of 0.1% formic acid and acetonitrile in the ratio of 30:70 (v/v), operated in isocratic mode. The analysis was carried out at a flow rate of 1.0 mL/min, with a detection wavelength of 262 nm and a total runtime of 3 minutes. The retention times were found to be 0.989 minutes for drospirenone and 1.878 minutes for estetrol, indicating a very rapid and efficient analytical method.

System suitability parameters such as theoretical plate count, tailing factor, and resolution were found to be within acceptable limits, confirming good chromatographic performance (Table 2, page 3). The method exhibited excellent specificity, with no interference observed from blank or excipients at the retention times of the analytes.

Linearity was established over the concentration range of 3–45 µg/mL for drospirenone and 14.2–213 µg/mL for estetrol, with correlation coefficients of 0.9992 and 0.9999, respectively. The regression equations obtained were $y = 18252.64x + 87.60$ for drospirenone and $y = 16149.69x + 351.33$ for estetrol, indicating excellent linearity and sensitivity of the method (Table 3, page 4).

Precision studies, including intra-day and inter-day analysis, showed %RSD values less than 2%, confirming the reproducibility of the method (Tables 4 and 5, page 5). Accuracy studies conducted at 50%, 100%, and 150% levels

demonstrated recovery values in the range of 98–100%, indicating high accuracy (Table 6, page 5).

Robustness studies performed by varying chromatographic parameters such as flow rate, mobile phase composition, and temperature showed that the %RSD values remained within acceptable limits, confirming the robustness of the method (Table 7, page 6).

Forced degradation studies were carried out under various stress conditions including acid, base, oxidative, reduction, and thermal degradation. The results (Table 8, page 6) indicated that both drugs underwent degradation under these conditions, with degradation ranging approximately between 11% and 16%. Importantly, no interference from degradation products was observed with the main peaks, confirming the stability-indicating capability of the method.

Overall, the developed RP-UPLC method was found to be rapid, sensitive, accurate, precise, robust, and stability-indicating. Due to its short runtime and high efficiency, the method is highly suitable for routine quality control and stability testing of drospirenone and estetrol in pharmaceutical formulations. [2]

Prathamesh Chaudhari et al. (2024).

A novel green analytical RP-HPLC method for the determination of drospirenone and its impurities was developed by Prathamesh Chaudhari et al. (2024). The method aimed to provide an environmentally friendly and efficient approach for the quantification of drospirenone in bulk and pharmaceutical dosage forms along with impurity profiling. Chromatographic separation was achieved using a BDS Hypersil C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and water in the ratio of 60:40 (v/v), operated in isocratic mode. The analysis was carried out at a flow rate of 1.0 mL/min, with UV detection at 271 nm and a total runtime of 10 minutes. The retention time of drospirenone was found to be approximately 4.7 minutes, indicating efficient separation (Table 2, page 5).

The method demonstrated excellent linearity over the concentration range of 10–60 µg/mL, with a correlation coefficient close to 0.999 and regression equation of y = 23774x + 17167, confirming the sensitivity of the method (Table 3, page 5). Precision studies, including intra-day and inter-day analysis, showed %RSD values less than 2%, indicating good reproducibility and reliability (Tables 4 and 5, pages 5–6).

Accuracy studies performed at three levels (80%, 100%, and 120%) showed recovery values ranging from 100.58% to 101.52%, demonstrating the high accuracy of the method (Table 6, page 6). Robustness studies conducted by varying parameters such as flow rate (±0.2 mL/min), temperature (±5°C), and wavelength (±2 nm) showed minimal variation in results, confirming the robustness of the method (Table 7, page 7).

A significant feature of this study is the impurity profiling of drospirenone. The method successfully identified and quantified impurities such as 7-hydroxymethyl drospirenone and drospirenone related compound A, as shown in chromatograms (pages 8–9). These impurities were effectively separated from the main drug peak without interference, confirming the specificity of the method.

Additionally, the greenness of the analytical method was evaluated using tools such as AGREE, GAPI, Complex GAPI, and HPLC-EAT. The AGREE score (approximately 0.89, shown in the pictogram on page 8) indicates that the method is environmentally friendly with reduced solvent consumption and minimal environmental impact.

Overall, the developed RP-HPLC method was found to be simple, accurate, precise, robust, eco-friendly, and suitable for routine quality control and impurity analysis of drospirenone in pharmaceutical formulations. [8]

Dhruvika Singh Chouhan and Anju Goyal (2023).

An efficient and validated RP-HPLC method for the simultaneous estimation of drospirenone and estetrol in tablet dosage form was developed by Dhruvika Singh Chouhan and Anju Goyal (2023). The chromatographic separation was achieved using a Kromasil C18 column (4.1 × 150 mm, 2.1 µm) with a mobile phase consisting of buffer (0.1% ortho-phosphoric acid with triethylamine, pH 4.5) and acetonitrile in the ratio of 55:45. The analysis was performed at a flow rate of 1.0 mL/min, with detection at 221 nm and column temperature maintained at 30°C. The method showed good separation with retention times of 2.408 minutes for drospirenone and 3.163 minutes for estetrol, indicating a rapid and efficient analytical procedure (as shown in optimized chromatograms on page 8).

System suitability parameters such as theoretical plate count (>3000), tailing factor (<2), and resolution (>3.5) confirmed good chromatographic performance (Table 1, page 8). Specificity studies indicated that there was no interference from blank or placebo at the retention times of the analytes, confirming the selectivity of the method (page 9).

The method exhibited excellent linearity over the concentration ranges of 1.5–9 µg/mL for drospirenone and 7.1–42.6 µg/mL for estetrol, with correlation coefficients of 0.999 for both drugs. The regression equations were found to be y = 13263x + 345.0 for drospirenone and y = 14243x + 1925 for estetrol, indicating strong linear relationships (Table 2, page 10).

Precision studies showed %RSD values of 0.2% for drospirenone and 1.1% for estetrol for system precision, while repeatability and intermediate precision studies also showed %RSD values below 2%, confirming excellent reproducibility (Tables 3–5, pages 10–11).

Accuracy studies performed at 50%, 100%, and 150% levels demonstrated mean recovery values of 100.58% for drospirenone and 99.43% for estetrol, which are within acceptable limits (98–102%), confirming the accuracy of the method (Tables 6 and 7, page 11–12).

The sensitivity of the method was confirmed with LOD values of 0.04 µg/mL for drospirenone and 0.54 µg/mL for estetrol, and LOQ values of 0.13 µg/mL and 1.63 µg/mL, respectively (Table 8, page 12). Robustness studies showed that small variations in chromatographic conditions did not significantly affect the results, with %RSD values remaining within acceptable limits (Table 9, page 12).

Forced degradation studies demonstrated that both drugs underwent maximum degradation under acidic conditions (~6%), followed by base and oxidative conditions, while minimal degradation was observed under thermal, UV, and hydrolytic conditions (Table 12, page 13). Importantly, no interference from degradation products was observed with the main peaks, confirming the stability-indicating capability of the method.

The assay of marketed formulation (Nextstellis tablets) showed average assay values of 100.23% for drospirenone and 99.21% for estetrol, indicating compliance with label claims (Tables 10 and 11, page 12–13). Overall, the developed RP-HPLC method was found to be simple, accurate,

precise, robust, sensitive, and suitable for routine quality control and stability studies of drospirenone and estetrol in combined dosage forms. [5]

S. Vinod and Y. Rajendraprasad (2022).

A validated stability-indicating RP-HPLC method for the simultaneous determination of estetrol and drospirenone in bulk and pharmaceutical dosage forms was developed by S. Vinod and Y. Rajendraprasad (2022). The chromatographic separation was carried out using a Waters Symmetry C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of 0.01N KH_2PO_4 buffer and acetonitrile in the ratio of 55:45 (v/v), delivered at a flow rate of 1.0 mL/min. The detection wavelength was set at 240 nm, and the total runtime of the method was approximately 6 minutes, indicating a relatively rapid analytical procedure (Abstract, page 1).

The method demonstrated good linearity over the concentration ranges of 3.55–21.3 µg/mL for estetrol and 0.75–4.5 µg/mL for drospirenone. As shown in the calibration data (Table 3.8, page 23), both drugs exhibited excellent correlation coefficients ($r^2 \approx 0.999$), confirming strong linear relationships between concentration and peak area.

Accuracy studies indicated high recovery values, with mean recoveries of approximately 100.17% for estetrol and 100.20% for drospirenone (Tables 3.9 and 3.10, pages 23–24), demonstrating the accuracy of the method. Precision studies showed %RSD values less than 2% for both drugs, confirming good repeatability and reproducibility (Table 2.11, page 24).

The method also demonstrated good sensitivity, with LOD and LOQ values determined based on signal-to-noise ratios (Tables 3.14 and 3.15, page 24). Robustness studies performed by varying flow rate, temperature, and mobile phase composition showed minimal changes in chromatographic parameters such as retention time, peak area, and tailing factor, confirming the robustness of the method (Tables 3.16–3.21, pages 24–25).

System suitability parameters, including retention time, peak area, and %RSD values, were found to be within acceptable limits, confirming the reliability of the analytical system (Table 3.22, page 25). The retention times were approximately 2.18 minutes for estetrol and 2.90 minutes for drospirenone, indicating effective separation (Table 3.22, page 25).

Forced degradation studies were carried out under various stress conditions including acidic, alkaline, oxidative, thermal, photolytic, and neutral environments. As shown in Tables 3.23 and 3.24 (page 25), both drugs exhibited moderate degradation under acidic and alkaline conditions (approximately 4–5%), while minimal degradation was observed under thermal, photolytic, and neutral conditions. Importantly, degradation products were well separated from the main peaks, confirming the stability-indicating capability of the method.

Overall, the developed RP-HPLC method was found to be simple, accurate, precise, robust, and stability-indicating. It is suitable for routine quality control and analysis of combined dosage forms containing estetrol and drospirenone, with no interference from excipients or degradation products. [3]

K. Vinutha et al. (2024).

A simple and validated RP-HPLC method for the simultaneous estimation of drospirenone and estetrol in bulk and tablet dosage forms was developed by K. Vinutha et al. (2024). The

chromatographic separation was achieved using a Kromosil C18 column (150 × 4.6 mm, 5 µm) with a mobile phase consisting of 0.01N KH_2PO_4 buffer and methanol in the ratio of 80:20 (v/v). The analysis was carried out at a flow rate of 1.0 mL/min with detection at 263 nm, and the column temperature was maintained at 30°C. The method showed efficient separation with retention times of 2.597 minutes for drospirenone and 2.133 minutes for estetrol, indicating a rapid analytical process (page 1 and chromatogram on page 3).

System suitability studies demonstrated satisfactory chromatographic performance, with theoretical plate counts above 5000, tailing factors around 1.2, and resolution values greater than 3.5, confirming the efficiency of the method (Table 1, page 7). Specificity studies revealed no interference from blank or placebo at the retention times of the analytes, indicating high selectivity.

Linearity was established over the concentration ranges of 1.5–9 µg/mL for drospirenone and 7.1–42.6 µg/mL for estetrol. As shown in the calibration data (Table 2, page 8), both drugs exhibited excellent correlation coefficients ($r^2 \approx 0.999$), with regression equations of $y = 361228x + 6065.6$ for drospirenone and $y = 341114x + 46689$ for estetrol, indicating strong linear relationships.

Precision studies, including system precision and repeatability, showed %RSD values of approximately 0.4% for both drugs, confirming excellent reproducibility (Tables 3 and 4, pages 9–10). Accuracy studies using the standard addition method demonstrated mean recovery values of 100.25% for drospirenone and 100.09% for estetrol, which fall within acceptable limits (Tables 5 and 6, page 10).

The sensitivity of the method was confirmed with LOD values of 0.01 µg/mL for drospirenone and 0.08 µg/mL for estetrol, and LOQ values of 0.02 µg/mL and 0.25 µg/mL, respectively (Table 7, page 10). Robustness studies indicated that small variations in flow rate, mobile phase composition, and temperature did not significantly affect the results, with %RSD values remaining within acceptable limits (Table 8, page 12).

Forced degradation studies conducted under acidic, basic, oxidative, thermal, photolytic, and neutral conditions demonstrated that both drugs showed maximum degradation under peroxide (oxidative) conditions (~6%), followed by acid and base conditions, while minimal degradation was observed under thermal, UV, and water conditions (Table 11, page 12). Importantly, degradation products were well separated from the main peaks, confirming the stability-indicating nature of the method.

The method was successfully applied to the assay of marketed formulation (Nextstellis tablets), where the percentage assay was found to be 100.17% for drospirenone and 99.64% for estetrol, indicating compliance with label claims (Tables 9 and 10, page 12). Overall, the developed RP-HPLC method was found to be simple, accurate, precise, robust, sensitive, and suitable for routine quality control and stability studies of drospirenone and estetrol in combined dosage forms. [6]

Syed Ismail Jabiullah et al. (2022).

A simple, precise, and validated RP-HPLC method for the simultaneous estimation of drospirenone and estetrol in bulk and pharmaceutical dosage forms was developed by Syed Ismail Jabiullah et al. (2022). The chromatographic separation was performed using an Agilent C18 column (150 × 4.5 mm, 3.5 µm) with an isocratic mobile phase consisting of orthophosphoric acid and acetonitrile in the ratio of 55:45. The analysis was carried out

at a flow rate of 1.0 mL/min with detection at 240 nm and a total runtime of approximately 6 minutes. The retention times were found to be 3.011 minutes for drospirenone and 2.403 minutes for estetrol, indicating efficient separation (as shown in chromatogram on page 4).

System suitability parameters such as plate count (>5000), tailing factor (<2), and resolution (>4) were within acceptable limits, confirming good chromatographic performance (Table 1, page 4). Specificity studies showed that no interference from blank or placebo was observed at the retention times of the analytes, indicating high selectivity (Table 2, page 4).

The method exhibited excellent linearity over the concentration ranges of 1.5–9 µg/mL for drospirenone and 7.1–42.6 µg/mL for estetrol. As shown in the calibration data (Table 3, page 5 and calibration curves on page 6), the regression equations were y = 11789x + 0.285 for drospirenone and y = 96131x + 8828 for estetrol, with correlation coefficients of 0.999, indicating strong linear relationships.

Accuracy studies performed using the standard addition method showed mean recovery values of 99.51% for drospirenone and 100.29% for estetrol, demonstrating the accuracy of the method (Tables 4 and 5, pages 6–7). Precision studies showed %RSD values of 0.3% for drospirenone and 0.8% for estetrol, confirming excellent repeatability and reproducibility (Table 6, page 7).

Robustness studies conducted by varying chromatographic parameters such as flow rate, mobile phase composition, and temperature showed that %RSD values remained within acceptable limits, indicating that the method is robust (Table 7, page 7–8).

The assay of marketed formulations showed percentage assay values of 99.87% for drospirenone and 100.07% for estetrol, confirming compliance with label claims (Table 8, page 8). The sensitivity of the method was confirmed with LOD values of 0.09 µg/mL for drospirenone and 0.36 µg/mL for estetrol, and LOQ values of 0.26 µg/mL and 1.10 µg/mL, respectively (Table 9, page 8).

Overall, the developed RP-HPLC method was found to be simple, accurate, precise, robust, and economical, making it highly suitable for routine quality control and analysis of drospirenone and estetrol in combined pharmaceutical dosage forms.[1]

Vignesh T et al. (2025).

A stability-indicating RP-HPLC method for the simultaneous estimation of estetrol and drospirenone in bulk and pharmaceutical dosage forms was developed by Vignesh T et al. (2025). The chromatographic separation was achieved using a Waters Kromasil C18 column (150 × 4.6 mm, 5 µm) with a mobile phase consisting of formic acid buffer and acetonitrile in the ratio of 70:30 (v/v). The analysis was carried out at a flow rate of 1.0 mL/min with UV detection at 265 nm. The retention times were found to be 2.228 minutes for estetrol and 2.859 minutes for drospirenone, demonstrating efficient and rapid separation (as shown in optimized chromatogram on page 6).

System suitability parameters such as plate count (>6500), tailing factor (~1.2), and resolution (~4.6) were found to be within acceptable limits, confirming good chromatographic performance (Table 1, page 7). Specificity studies revealed no interference from blank or placebo at the retention times of the analytes, indicating high selectivity of the method (page 7).

The method showed excellent linearity over the concentration ranges of 3–18 µg/mL for drospirenone and 14.2–85.2 µg/mL for estetrol. As presented in the calibration data (Table 2, page 8), the regression equations were y = 69652x + 1303.1 for drospirenone and y = 62844x + 50224 for estetrol, with correlation coefficients of 0.999, indicating strong linear relationships.

Precision studies, including system precision, repeatability, and intermediate precision, showed %RSD values less than 2% (typically between 0.2–0.8%), confirming excellent reproducibility of the method (Tables 3–5, pages 9–10). Accuracy studies demonstrated mean recovery values of 99.52% for drospirenone and 99.89% for estetrol, indicating high accuracy (Tables 6 and 7, page 11).

The sensitivity of the method was confirmed with low LOD and LOQ values. The LOD values were 0.03 µg/mL for drospirenone and 0.20 µg/mL for estetrol, while LOQ values were 0.08 µg/mL and 0.61 µg/mL, respectively (Table 8, page 12).

Robustness studies indicated that small variations in chromatographic conditions such as flow rate, mobile phase composition, and temperature did not significantly affect the results, with %RSD values remaining within acceptable limits (Table 9, page 12).

Assay results of marketed formulations showed mean assay values of 99.71% for drospirenone and 100.22% for estetrol, confirming compliance with label claims (Tables 10 and 11, page 13).

Forced degradation studies demonstrated that both drugs remained stable under various stress conditions, with minimal degradation observed. The highest degradation was observed under acidic conditions (6.22% for drospirenone and 5.20% for estetrol), while the least degradation occurred under neutral (water) conditions (Table 12, pages 14–15). Importantly, degradation products were well separated from the main peaks, confirming the stability-indicating nature of the method.

Overall, the developed RP-HPLC method was found to be simple, precise, accurate, robust, sensitive, and stability-indicating. It is highly suitable for routine quality control, stability testing, and regulatory compliance in pharmaceutical analysis of combined dosage forms containing estetrol and drospirenone. [9]

Comparative analysis with Parameters :

Comparative Analysis of Chromatographic Conditions for Drospirenone and Estetrol

Table 1: Papers 1–4

Parameter	Jabiullah et al. (2022)	Vinod et al. (2022)	Swathi et al. (2023)	Chouhan et al. (2023)
Column	C18 Column	ACE-EPS C18	Agilent C18	Kromasil 150
Mobile Phase	OPA:ACN (55:45)	0.1% OPA:ACN (60:40)	0.1N KH2PO4:ACN (50:50)	Buffer:ACN (55:45)
pH	NR	Acidic	NR	4.5
Flow Rate (mL/min)	1.0	1.0	1.0	1.0

Column Temperature (°C)	Ambient	Ambient	30	30
Injection Volume (µL)	10	10	NR	NR
Detection Wavelength (nm)	PDA	226	263	221
Rt Estetrol (min)	2.403	~2.4	2.268	3.163
Rt Drospirenone (min)	3.011	~3.0	2.698	2.408

Table 2: Papers 5–8

Parameter	Vinutha et al. (2024)	Rachapudi et al. (2024)	Vignesh et al. (2025)	Rafi Syed et al. (2021)
Column	Kromosil C18	Waters SunFire C18	Waters Kromasil C18	Luna C18
Mobile Phase	0.01N KH2PO4 : Methanol (80:20)	0.01N (NH4)3PO4 : ACN (70:30)	Formic Acid Buffer : ACN (70:30)	0.1% Formic Acid : ACN (30:70)
Ph	OPA Adjusted	6.8	Acidic	Acidic
Flow Rate (mL/min)	1.0	1.2	1.0	1.0
Column Temperature (°C)	30	Ambient	Ambient	Room Temperature
Injection Volume (µL)	NR	20	NR	NR
Detection Wavelength (nm)	263	265	265	262
Retention Time of Estetrol (min)	2.134	~2.2	2.228	< 2.0
Retention Time of Drospirenone (min)	2.597	~2.7	2.859	< 3.0

Various RP-HPLC and UPLC methods have been reported for the simultaneous estimation of Drospirenone and Estetrol in pharmaceutical dosage forms. Most of the reported methods employed reversed-phase C18 columns due to

their excellent retention characteristics and compatibility with moderately non-polar compounds. Syed Ismail Jabiullah et al. (2022) developed a method using a C18 column with a mobile phase consisting of orthophosphoric acid and acetonitrile (55:45 v/v). The method achieved retention times of 2.403 min for Estetrol and 3.011 min for Drospirenone, demonstrating satisfactory resolution and peak symmetry. Similarly, S. Vinod et al. (2022) utilized an ACE-EPS C18 column with 0.1% orthophosphoric acid and acetonitrile (60:40 v/v), obtaining efficient chromatographic separation within a short run time. [1,3,4,5]

L. Swathi et al. (2023) developed a stability-indicating RP-HPLC method using an Agilent C18 column (150 × 4.6 mm, 5 µm) and a mobile phase comprising 0.1 N KH_2PO_4 buffer and acetonitrile (50:50 v/v). The method was operated at a flow rate of 1.0 mL/min and a column temperature of 30°C, with UV detection at 263 nm. Retention times of 2.268 min and 2.698 min were observed for Estetrol and Drospirenone, respectively. Dhruvika Singh Chouhan et al. (2023) employed a Kromasil column with a buffer system adjusted to pH 4.5. The chromatographic conditions provided retention times of 3.163 min for Estetrol and 2.408 min for Drospirenone, indicating adequate selectivity and separation efficiency. [6,7]

Recent studies have further optimized chromatographic parameters to improve analysis time and sensitivity. K. Vinutha et al. (2024) used a Kromosil C18 column with 0.01 N KH_2PO_4 and methanol (80:20 v/v) as the mobile phase. Detection was performed at 263 nm, yielding retention times of 2.134 min for Estetrol and 2.597 min for Drospirenone. Geetha Rani Rachapudi et al. (2024) utilized a Waters SunFire C18 column with ammonium phosphate buffer and acetonitrile (70:30 v/v) at pH 6.8 and a flow rate of 1.2 mL/min. The method provided rapid separation with retention times around 2.2 min for Estetrol and 2.7 min for Drospirenone.

Vignesh T et al. (2025) developed a stability-indicating RP-HPLC method using a Waters Kromasil C18 column and a formic acid buffer-acetonitrile mobile phase (70:30 v/v). Detection was carried out at 265 nm, producing retention times of 2.228 min for Estetrol and 2.859 min for Drospirenone. Among all reported methods, the UPLC method developed by Rafi Syed and Rambabu Kantipudi (2021) demonstrated the highest efficiency. The method utilized a Luna C18 column (100 × 2.6 mm, 1.6 µm) with a mobile phase consisting of 0.1% formic acid and acetonitrile (30:70 v/v). Detection was performed at 262 nm, and complete separation of both analytes was achieved within 3 minutes, significantly reducing analysis time compared to conventional HPLC methods. [2,9]

Overall, the reviewed studies indicate that C18 stationary phases, acetonitrile-based mobile phases, acidic buffer systems, and flow rates around 1.0 mL/min provide optimal chromatographic performance for the simultaneous estimation of Drospirenone and Estetrol. Detection wavelengths between 262 and 265 nm were found to offer maximum sensitivity and specificity. The retention times reported in the literature generally ranged from 2.1 to 3.2 minutes, confirming the suitability of these methods for routine quality control and pharmaceutical analysis.

CONCLUSION

The present review summarizes and critically evaluates the various analytical methods reported for the simultaneous estimation of Drospirenone and Estetrol in bulk drugs and pharmaceutical dosage forms. Most of the reported methods employ reversed-phase chromatographic

techniques using C18 stationary phases, which provide excellent selectivity, peak symmetry, and reproducibility. Acetonitrile-based mobile phases combined with phosphate, formate, or orthophosphoric acid buffers were predominantly utilized to achieve efficient separation of the analytes.

The reviewed methods demonstrated satisfactory validation characteristics in accordance with ICH guidelines, including excellent linearity, accuracy, precision, robustness, specificity, and sensitivity. Detection wavelengths ranging from 221 to 265 nm and retention times generally below 3.5 minutes enabled rapid and reliable analysis. Among the reported methods, UPLC techniques offered superior efficiency, reduced solvent consumption, shorter run times, and enhanced sensitivity compared to conventional HPLC methods.

Recent advancements have also focused on stability-indicating and green analytical approaches, highlighting the growing emphasis on environmentally sustainable analytical procedures. Overall, the available RP-HPLC and UPLC methods are suitable for routine quality control, stability studies, and regulatory analysis of Drospirenone and Estetrol in pharmaceutical formulations. Future research should focus on the development of more eco-friendly, cost-effective, and QbD-based analytical methods that further improve method robustness, sustainability, and analytical performance while complying with modern regulatory requirements.

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