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Simultaneous Determination of Gestodene and Ethinyl Estradiol in Contraceptive Formulations by RP-HPLC

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ABSTRACT

A rapid and simple HPLC method for the determination of gestodene (GTD) and ethinyl estradiol (ETE) in oral contraceptive formulations was developed and validated. Separation of components was achieved on Waters Symmetry Shield Rp-18 column. Isocratic elution with a mobile phase consisting of methanol and water in a volume percent ratio 80:20 at a flow rate 1.0 mL/min was employed. The components were detected by UV detection at 220 nm. Ciproterone acetate was used as an internal standard (IS). Linear concentration range for GTD was between 0.01 and

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0.2 mg/mL and for ETE between 0.005 and 0.08 mg/mL. The detection limit was 2.3 μg/mL for GTD and 0.80 μg/mL for ETE. Mean analytical recovery in determination of GTD and ETE in “Mirelle” (Schering, FRG) tablets was 102.8% for GTD and 101.3% for ETE. Thus, the proposed method is applicable for routine determination of GTD and ETE in contraceptive formulations.

Key Words: Gestodene; Ethinyl estradiol; RP-HPLC; Contraceptive formulations.

INTRODUCTION

Gestodene (17α-13-ethyl-17-hydroxy-18,19-dinopregn-4,15-dien-20-yn-3-one, GTD) belongs to the third generation of progestogens which in combination with ethinyl estradiol (ETE) finds use as a mono-phase lower-dose contraceptive. So far HPLC, electrokinetic capillary chromatography, radio-immunoassay, and voltammetry were used for the determination of GTD in pharmaceutical formulations and biological samples.

Ethinyl estradiol (17α-19-norpregna-1,3,5(10)-trien-20-yn-3,17-diol) finds use as estrogen component in oral contraceptive formulations. Ethinyl-estradiol was determined by spectrophotometry, micellar electrokinetic chromatography, liquid chromatography and gas chromatography-mass spectrometry methods. Modern pharmacopoeia recommend the determination of ETE by HPLC method using either acetonitrile-water or methanol-water mobile phase. The simultaneous determination of both GTD and ETE in combined contraceptives was described in literature by applying micellar electrokinetic chromatography, spectrophotometry, and derivative spectrophotometry. Different methods for the determination of GTD and ciproterone acetate have been assayed. Since pharmacopoeias do not describe the suitable method for the concurrent determination of GTD and ETE in pharmaceutical formulations, in the present work we developed
rapid and accurate HPLC method for the simultaneous determination of GTD and ETE in tablets as an alternative method.

EXPERIMENTAL

Reagents

Analytical standards of GTD and ETE were obtained from Schering (Berlin) FRG as well as the pharmaceutical dosage form—"Mirelle" tablets. Declared quantities of hormones in tablets were 60 μg of GTD and 15 μg ETE per tablet. Water used for dissolution was doubly distilled. Methanol (HPLC analytical grade) was obtained from Merck (Darmstadt), FRG. Ciproterone acetate was used as an internal standard (IS).

Instruments

Chromatographic System

Chromatographic system, Agilent series 1100 (Waldbron, Germany) consisted of a binary pump, column thermostat, diode array detector and autosampler, was used for chromatographic analysis. The software, ChemStation (Agilent) was used for acquisition and processing of data.

Chromatographic Conditions

The HPLC separation was achieved on a reversed phase column Waters (Milford, USA)—SymmetryShield Rp 18. Column dimensions were 150 × 4.6 mm i.d., with particle size 5 μm. Mixture of methanol and water in a volume ratio 80 : 20 v/v% was used as a mobile phase without the addition of buffer. Mobile phase was vacuum filtered through 0.22 μm PTFE filter. Flow rate of the mobile phase was 1.0 mL/min. Injected volume of sample was 10 μL. The detector wavelength was set at 220 nm. The analysis was performed at ambient temperature.

Solutions

Stock solutions of GTD and ETE were separately prepared by direct weighing of analytical standards and their dissolving in a mobile phase. The series of working standard solutions for calibration graph were prepared by
diluting appropriate aliquots of the stock solution with the mobile phase, to yield the concentration of GTD in the range of 0.005–0.5 mg/mL and of ETE, 0.001–0.1 mg/mL. The obtained solutions were mixed in such a way that the calibration graph for the GTD was obtained by varying the GTD concentration and keeping the ETE concentration constant, while the calibration graph for ETE was obtained by varying the ETE concentration and keeping the GTD concentration constant. To each solution ciproterone acetate was added so that its concentration was always the same—0.0996 mg/mL. Each standard solution for the calibration graph was injected in triplicate. Average peak area ratio of analyte to IS (ciproterone acetate) was used in regression analysis. To test the hypothesis that the data used in linear model are homoskedastic (set of estimated variances of responses is homogeneous) the Cohran’s test\(^{[18]}\) was applied to the chromatographic data. The test is based on the distribution of the random variable \(G_{\text{exp}} = s_{\text{max}}^2(Y)/\sum s^2(Y_i)\), where \(s_{\text{max}}^2(Y)\) is the highest obtained variance of the \(Y\) response (i.e., peak area ratio). The variable \(G\) is function of number of replications, \(n\), and total number of measurements, \(k\), so as if the relationship \(G_{\text{exp}} < G_k(n - 1, k)\) is valid the hypothesis about homoskedasticity is true. At significance level \(\alpha = 0.05\), \(n = 3\), and \(k = 12\), \(G_{0.05}(2, 12) = 0.392\) and since experimentally obtained value \(G_{\text{exp}} = 0.306\) the tested hypothesis is accepted.

Sample Preparation

An amount of powdered tablets, equivalent to ca. 1200 μg of GTD and 300 μg of ETE, was dissolved in 7.0 mL of the mobile phase, sonicated for 15 min in an ultrasonic bath (Bandelin Sonorex Super, model RK 512H). The solution was centrifuged for 5 min at 3000 rpm; the supernatant was filtered through 0.22 μm PTFE filter, and then diluted with the mobile phase in the volumetric flask, to yield the final concentration of 120 μg/mL of GTD and 30 μg/mL of ETE. Three different aliquots of this solution were used in the same procedure as for the calibration graph. The concentration read from the calibration curve was multiplied with the dilution factor to obtain the final concentration of the drug.

Precision, Limit of Detection, and Limit of Quantification

Blank mobile phase was spiked with different volumes of standard solutions of GTD and ETE with the addition of IS. Samples were processed in replicates (\(n = 3\)) and subjected to HPLC analysis. The precision was
calculated as \(\%CV = (\text{SD/mean}) \times 100\), (SD = standard deviation of response) while limit of detection (LOD) was calculated as: \(^{19}\)

\[
\text{LOD} = 3.3 \frac{s_b}{a}
\]

where \(s_b\) is the standard deviation of y-intercept of the calibration line and \(a\) is the slope of the calibration line. Limit of quantification (LOQ) was calculated using the equation:

\[
\text{LOQ} = 10 \frac{s_b}{a}
\]

### Recovery

Recovery study was performed by spiking test portions with the standard solutions of either GTD or ETE with the addition of IS. The recovery was calculated through the formula: \(^{20}\)

\[
\%\text{Recovery} = \frac{\text{CF} - \text{CU}}{\text{CA}} \times 100
\]

where CF is the total concentration of analyte measured in spiked sample, CU is the concentration of the analyte measured in the unfortified test portion and CA is the concentration of the added analyte (GTD or ETE).

### Reproducibility

No variations in the retention times were observed using mobile phases prepared on different days. The column-to-column reproducibility was evaluated by injecting the samples on two columns from the same manufacturer, containing the same brand of packing material and on two columns from the different manufacturers but containing the same brand of the packing material. Only slight variations in retention times (within 1–2\%) were observed.

### RESULTS AND DISCUSSION

A reversed phase isocratic liquid chromatography proved its suitability for the simultaneous determination of GTD and ETE in tablets. It has been calculated as \(\%CV = (\text{SD/mean}) \times 100\), (SD = standard deviation of response) while limit of detection (LOD) was calculated as: \(^{19}\)
experimentally established that use of a mobile phase consisting of methanol and water in a volume percent ratio 80 : 20 leads to the excellent separation of analytes from the IS. Chromatographic characteristics of the peaks obtained from solutions prepared using standards of the drugs are summarized in Table 1.

Ciproterone acetate (IS) is clearly separated from both analytes (retention time 3.90 min). To apply the proposed method for the analysis of hormone dosage forms the method was validated for selectivity, linearity, accuracy, precision, LOQ, and LOD. Selectivity of the method was examined by taking chromatograms of the synthetic solution containing standard GTD, ETE, and IS and tablets solution with the addition of IS. Identical values for retention times and peak areas were obtained thus indicating that tablet excipients (microcrystalline cellulose, lactose, magnesium stearate, polyethylene glycol, titanium dioxide, iron oxide, and wax), degradation products and impurities do not interfere with the determination. Also, chromatograms of aged and freshly prepared samples showed no interfering peaks from degradation products. Linearity of the method was examined by analysis of peak area ratio of analyte to that of the IS, as a function of the concentration ratio of analyte to IS. For each point in the graph three replications were applied. Results of validation are given in Table 2.

Mobile phase (methanol : water = 80 : 20 v/v%) was chosen both for the extraction of hormones from tablets and as a solvent. Accuracy of the method was examined by carrying out "recovery" test in determination of GTD and ETE in "Mirelle" tablets. The obtained values are given in Table 3 while representative chromatogram of the tablet solution is shown in Fig. 1.

Nominal quantities of GTD and ETE in "Mirelle" tablets were 60 μg and 15 μg respectively, per tablet. The precision of the HPLC system was examined by using the coefficient of variation (CV) of the peak areas for 10 injections of the three standard solutions containing 120%, 100%, and 80% of hormones relative to their nominal quantity in tablets, per milliliter of

Table 1. Chromatographic parameters for GTD and ETE separation on SymmetryShield Rp18 column.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gestodene</th>
<th>Ethinyl estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>3.22</td>
<td>4.74</td>
</tr>
<tr>
<td>Capacity factor</td>
<td>1.71</td>
<td>2.56</td>
</tr>
<tr>
<td>Selectivity</td>
<td></td>
<td>1.50</td>
</tr>
<tr>
<td>Resolution</td>
<td></td>
<td>7.20</td>
</tr>
</tbody>
</table>
solution. The obtained maximum value for CV of 1.2%, shows that the precision of the method as applied to tablets is adequate. In conclusion it may be said that the proposed method is precise, sensitive and accurate so that it can be used as a standard pharmacopoeial method for the determination of GTD and ETE.

### Table 2. Validation of the HPLC method for determination of GTD and ETE

<table>
<thead>
<tr>
<th>Validated parameter</th>
<th>Gestodene</th>
<th>Ethinyl estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range of concentrations</td>
<td>0.01–0.2 mg/mL</td>
<td>0.005–0.08 mg/mL</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$Y = 1.692X + 0.019$</td>
<td>$Y = 1.870X + 0.002$</td>
</tr>
<tr>
<td>Coefficient of correlation ($r^2$)</td>
<td>0.99982</td>
<td>0.99985</td>
</tr>
<tr>
<td>Error on slope</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Error on intercept</td>
<td>0.012</td>
<td>0.004</td>
</tr>
<tr>
<td>Standard error of the estimation</td>
<td>0.016</td>
<td>0.007</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>2.3 μg/mL</td>
<td>0.80 μg/mL</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>7.2 μg/mL</td>
<td>9.6 μg/mL</td>
</tr>
</tbody>
</table>

### Table 3. Recovery test in determination of GTD and ETE in “Mirelle” tablets (nominal quantity 60 μg and 15 μg per tablet, respectively).

<table>
<thead>
<tr>
<th>Run</th>
<th>Gestodene</th>
<th>Ethinyl estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found (mg/tablet)</td>
<td>Recovery</td>
</tr>
<tr>
<td>1</td>
<td>0.0618</td>
<td>103.0</td>
</tr>
<tr>
<td>2</td>
<td>0.0619</td>
<td>103.2</td>
</tr>
<tr>
<td>3</td>
<td>0.0617</td>
<td>102.8</td>
</tr>
<tr>
<td>4</td>
<td>0.0615</td>
<td>102.5</td>
</tr>
<tr>
<td>5</td>
<td>0.0616</td>
<td>102.7</td>
</tr>
<tr>
<td>Average</td>
<td>0.0617</td>
<td>102.8</td>
</tr>
<tr>
<td>SD</td>
<td>$1.58 \times 10^{-4}$</td>
<td>0.27</td>
</tr>
<tr>
<td>CV%</td>
<td>0.25</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Figure 1. Chromatogram of solution of "Mirelle" (Schering, FRG) tablets. Ciproterone acetate was added as IS.
of GTD and ETE in tablets. The advantages of the proposed method involve simple procedure for sample preparation and relatively short time of analysis.

REFERENCES


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