Determination of Eprosartan Mesylate and Hydrochlorothiazide in Tablets by Derivative Spectrophotometric and High-Performance Liquid Chromatographic Methods

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Two new simple and selective assay methods have been presented for the analysis of eprosartan mesylate (EPR) and hydrochlorothiazide (HCT) in pharmaceutical formulations. The first method is based on first-derivative ultraviolet spectrophotometry with zero-crossing measurements at 246 and 279 nm for EPR and HCT, respectively. The assay was linear over the concentration ranges 3.0–14.0 µg/mL for EPR and 1.0–12.0 µg/mL for HCT. The quantification limits for EPR and HCT were found to be 1.148 and 0.581 µg/mL, respectively, while the detection limits were 0.344 µg/mL for EPR and 0.175 µg/mL for HCT. The second method involved isotropic reversed-phase liquid chromatography using a mobile phase composed of acetonitrile–10 mM phosphoric acid (pH 2.5) (40:60, v:v). Olmesartan was used as internal standard and the substances were detected at 272 nm. The linearity ranges were found to be 0.5–30 and 0.3–15.0 µg/mL for EPR and HCT, respectively. The limits of detection were found to be 0.121 µg/mL for EPR and 0.045 µg/mL for HCT. The limits of quantification were found to be 0.405 and 0.148 µg/mL for EPR and HCT, respectively. The proposed methods were successfully applied to the determination of commercially available tablets with a high percentage of recovery and good accuracy and precision.

Introduction

Eprosartan mesylate (EPR) (Figure 1A), monomethanesulfonate of (E)-2-buty1-1-(p-carboxybenzyl)-a-2-thienylmethylimidazole-5-acrylic acid, is a highly selective, nonpeptide angiotensin-II antagonist. The compound has been shown to inhibit angiotensin-II, including vasoconstriction in preclinical species, and to cause reductions in systolic and diastolic blood pressure at peak effect after dosing in clinical patients. It belongs to the ARA-II family. These are safe and effective agents for the treatment of hypertension and heart failure, either alone or in combination with diuretics (1, 2). The combination of EPR and hydrochlorothiazide (HCT) can be effectively and safely used in patients (3). Two methods have been reported for the determination of EPR in pharmaceutical preparations: ultraviolet (UV)-spectrophotometry (4) and high-performance liquid chromatography (HPLC) (5).

HCT (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide) (Figure 1B) is a potent orally diuretic and antihypertensive agent related to chlorothiazide. Several analytical methods have been published on the determination of HCT in tablets using flow injection (6), spectrophotometric (7–9), densitometric (10), HPLC (7–9, 10–13), electrophoretic (14, 15) and polarographic (16) methods.

In our literature survey, only one high-performance thin layer chromatography (HPTLC) method was observed for simultaneous analysis of these drugs in pharmaceutical preparations (17). HPTLC has the advantage of operation simplicity and low cost. However, HPLC is a more commonly used method in quality control laboratories because of its high sensitivity and precision. The derivative UV-spectrophotometric method is also very simple and does not require any reagent, pH-adjustment or extraction procedure. For this purpose, a zero-crossing first-derivative spectrophotometric method and an HPLC method were developed for analysis of EPR and HCT in mixtures without prior separation. Additionally, the proposed methods are shown to be useful in determination of both drugs in combination tablet formulations.

Experimental

Apparatus

Spectrophotometric measurements were performed by using a Shimadzu UV-160 A spectrophotometer with 1-cm glass cells.

The HPLC analyses were performed on a Thermo Separation Products Liquid Chromatograph that consisted of a P4000 solvent delivery system equipped with a Rheodyne injection valve with a 20-µL loop, a UV3000 detector set at 272 nm and an SN4000 automation system software. Chromatographic separation was achieved isocratically at a temperature of 30°C on an ACE 5 CN column (4.6 mm I.D. × 200 mm, 5 μm; Aberdeen, Scotland). The mobile phase was composed of acetonitrile–10 mM phosphoric acid (pH 2.5; 40:60, v:v) with a flow rate of 1.0 mL/min.

Reagents and solutions

EPR was supplied by Solvay Pharmaceuticals (Netherlands). HCT was supplied Abdi Ibrahim Pharmaceuticals (Istanbul, Turkey). Their pharmaceutical preparation Teveten Plus tablet, containing 600 mg of EPR and 12.5 mg of HCT per tablet, were obtained from a local drugstore. All chemicals and reagents were of analytical-reagent grade.

For both methods, portions (100 mg each) of standard EPR (calculated as a base) and HCT were weighed and transferred to 100-mL volumetric flasks and dissolved in methanol, and further dilutions were made with methanol at concentrations of 100 µg/mL each to obtain standard solutions of EPR and HCT. Before measurements, final dilutions were made with the acetonitrile–water to 50:50 and 40:60 (for derivative spectrometry and HPLC methods, respectively).
Internal standard (IS) stock solution was prepared by dissolving 50.0 mg of olmesartan in 50 mL of methanol. The stock solutions were stored at 4°C and were stable for a month.

**General procedure**

**Derivative spectrophotometric method**
Different aliquots of drug solution (0.05–0.7 mL) were transferred to 5-mL volumetric flasks to provide a final concentration range of 1.0–10.0 µg/mL for HCT and 3.0–14.0 µg/mL for EPR, and the volume was diluted to final volume with acetonitrile–water (50:50).

The first derivative spectra of these standard solutions were scanned against an acetonitrile–water (50:50) blank between 200 and 400 nm. The values of the derivative amplitudes at 246 nm and 279 nm were measured for the determination of EPR and HCT, respectively. The concentrations of each compound versus their derivative amplitudes were plotted to establish calibration graphs.

**HPLC method**
Standard solutions of EPR and HCT were prepared by dilution of the stock solutions with acetonitrile–water (40:60, v/v).

The final concentrations of each drug were between 0.5–30 and 0.3–15.0 µg/mL for EPR and HCT, respectively. These standard solutions also contained an IS at 50 µg/mL. The chromatograms were evaluated on the basis of EPR–IS or HCTZ–IS ratios of the peak areas.

**Assay procedure for tablets**
For the spectrophotometric method, 20 tablets were weighed and finely powdered. Powder equivalent to 60 mg EPR and 1.25 mg HCT was accurately weighed and transferred to a 100-mL volumetric flask. Methanol (75 mL) was transferred to the volumetric flask and then extraction was performed mechanically for 20 min and sonication for 20 more min. The dilution was made with methanol to reach a solution containing 600 µg/mL EPR and 12.5 µg/mL HCT (Solution 1), which was used for the determination of HCT. One milliliter of this solution was transferred to a 100-mL volumetric flask. The volume was diluted with acetonitrile–water, 50:50 and 40:60 (for derivative spectrometry and HPLC methods, respectively) to a final volume to have a solution containing 6 µg/mL EPR and 0.125 µg/mL HCT (Solution 2), which was used for the determination of EPR only. The difference between the amount of EPR and HCT in the tablet formulation was very high;
therefore, measurements were performed at different dilution levels. First, HCT was measured at a low dilution level, then EPR was measured at a higher dilution level.

Results and Discussion

Method Development

A zero-crossing first-derivative spectrophotometric method and an HPLC method were developed for the analysis of EPR and HCT in combination tablet preparations.

Direct UV-absorption measurements were found to be inapplicable to the analysis of EPR and HCT in simultaneous analysis because of the spectral interference (Figure 2). On the other hand, derivative spectrophotometry based on a mathematical transformation of the zero-order curve into the derivative spectra can overcome this problem (18–23). The EPR and HCT were prepared in different solvents (acetonitrile, methanol and water) and mixtures of the solvents. The best results were found with acetonitrile–water (50:50). Using memory channels, the first to fourth order derivative spectra were overlapped. The first order derivative spectrum was selected based on available zero crossing points. As shown in Figure 3C, the wavelength 243 nm was selected for the determination of EPR (where the derivative response for HCT was zero) and 279 nm was selected for the determination of HCT (where the derivative response for EPR was zero).

As a new alternate method for analysis of these drugs and to verify the results of the developed UV-derivative spectrophotometric method, an HPLC method was also developed. For a good chromatographic separation, some parameters were tested. A C18 and a reversed-phase CN column were used for the column selection. Trials showed that the reversed-phase CN column gave symmetrical and sharp peaks. Acidic and aqueous mobile phase were tested, and acidic mobile phase provided good results. Therefore, o-phosphoric acid solution (10 mM) was preferred as the acidic solution. Acetonitrile was chosen as the organic modifier. A mobile phase composition of acetonitrile–phosphoric acid (pH: 2.5), 40:60 (v/v) at a flow rate of 1.0 mL/min was used to achieve a good resolution. Olmesartan, telmisartan, valsartan, irbesartan were tested as IS, and olmesartan was chosen as IS. For quantitative analytical purposes, the wavelength was set at 272 nm (Figure 2). Retention times of the drugs obtained under these conditions were 1.02, 1.03, 1.04, and 1.05 min for olmesartan, telmisartan, valsartan, and irbesartan, respectively. For quantitative purposes, the wavelength was set at 272 nm for both methods. Figure 2 shows the overlaid zero-order spectra of eprosartan and hydrochlorothiazide in acetonitrile–water (50:50).
conditions are 4.2, 5.9 and 7.5 for HCT, EPR, and IS, respectively (Figure 4).

**Method validation**

**Linearity range**

For derivative spectrophotometry, calibration curves were constructed by plotting two-dimensional (2D) values against corresponding concentrations in the range of 1.0–10.0 and 3.0–14.0 μg/mL for HCT and EPR, respectively. The regression equations were calculated as $4D_{243} = 0.0086C + 0.0025 (r^2 = 0.9995)$ for HCT and $4D_{279} = 0.0054C - 0.0025 (r^2 = 0.9997)$ for EPR.

For HPLC analysis, the equations of the calibration curves were obtained from linear regression analysis of the peak area ratios of EPR to IS or HCT to IS versus the concentration of related substance. The linearity ranges were found to be 0.5–30 and 0.3–15.0 μg for EPR and HCT, respectively. Regression equations of the calibration curves for EPR and HCT were calculated as $AEPR/AIS = 0.1214C + 0.0125 (r^2 = 0.9992)$ and $AHCT/AIS = 0.0593C + 0.0195 (r^2 = 0.9999)$, respectively.

**LOD and LOQ**

The limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula LOD or LOQ = $\kappa SDa/b$, where $\kappa = 3$ for LOD and 10 for LOQ, $SDa$ is the standard deviation of the intercept, and $b$ is the slope. For derivative spectrophotometric method, the LOD were found to be 0.345 μg/mL for EPR and 0.174 μg/mL for HCT, and the LOQ were found to be 1.148 μg/mL for EPR and 0.581 μg/mL for HCT. For the HPLC method, the LOD for EPR and HCT were found to be 0.121 and 0.045 μg/mL, respectively, and the LOQ were 0.405 μg/mL for EPR and 0.148 μg/mL for HCT.

The results of some analytical parameters of the proposed methods are given in Table I.

**Precision**

The inter-day and intra-day precision were examined by analysis of drugs for the same day and seven consecutive days (each $n = 5$). The relative standard deviation (RSD) values found for the developed methods were 0.64–1.18% for intra-day precision and 0.95–1.29% for inter-day precision, indicating good precision.

**Recovery**

To check the accuracy of the proposed methods, the standard addition technique was applied. A different amount of pure sample solution was added to four different concentrations of the standard drug solution and assayed. The percent recovery of the added standard to the assay samples was calculated from:

$$\text{Recovery}\% = \left[\frac{(C_t - C_u)/C_a}{C_t/C_a}\right] \times 100$$

Where $C_t$ is the total concentration of the analyte found; $C_u$ is the concentration of the analyte present in the formulation; and $C_a$ is the concentration of the pure analyte added to the formulation. The results of analysis of the commercial dosage forms and the recovery study are shown in Table II. The average percent recoveries for all methods were quantitative (99.80–101.20%), indicating good accuracy of the methods.
Table II
Results of Recovery Studies by Standard Addition Method

<table>
<thead>
<tr>
<th>Amount removed (μg/mL)*</th>
<th>Amount added (μg/mL)</th>
<th>Total amount found† (Mean ± S.D.c)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For EPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>5.003 ± 0.037</td>
<td>100.10</td>
<td>0.73</td>
</tr>
<tr>
<td>7.0</td>
<td>9.002 ± 0.068</td>
<td>100.03</td>
<td>0.75</td>
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</tr>
<tr>
<td>12.0</td>
<td>14.125 ± 0.128</td>
<td>101.04</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>For HCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>5.009 ± 0.045</td>
<td>100.30</td>
<td>0.90</td>
</tr>
<tr>
<td>7.0</td>
<td>8.996 ± 0.0723</td>
<td>99.94</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>12.104 ± 0.089</td>
<td>101.04</td>
<td>0.74</td>
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<tr>
<td>HPLC method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For EPR</td>
<td>0.50</td>
<td>10.506 ± 0.087</td>
<td>101.20</td>
<td>0.83</td>
</tr>
<tr>
<td>10.0</td>
<td>2.0</td>
<td>11.996 ± 0.112</td>
<td>99.80</td>
<td>0.93</td>
</tr>
<tr>
<td>12.0</td>
<td>22.115 ± 0.187</td>
<td>100.96</td>
<td>0.85</td>
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<tr>
<td>20.0</td>
<td>30.214 ± 0.401</td>
<td>101.07</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>For HCT</td>
<td>0.3</td>
<td>5.304 ± 0.065</td>
<td>101.33</td>
<td>1.23</td>
</tr>
<tr>
<td>5.0</td>
<td>1.5</td>
<td>6.507 ± 0.087</td>
<td>100.47</td>
<td>1.34</td>
</tr>
<tr>
<td>6.0</td>
<td>11.012 ± 0.093</td>
<td>100.20</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>15.0</td>
<td>20.123 ± 0.115</td>
<td>100.82</td>
<td>0.57</td>
<td></td>
</tr>
</tbody>
</table>

*Teveten Plus tablet, containing 600 mg of EPR and 12.5 mg of HCT per tablet.
† Five independent analyses.
‡ Teveten Plus tablet, containing 600 mg of EPR and 12.5 mg of HCT per tablet.

Table III
Results from Robustness Experiments for HPLC Method

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPR</td>
<td>HCT</td>
<td>EPR</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>0.95</td>
<td>100.73</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>100.83</td>
<td>1.29</td>
</tr>
<tr>
<td>Mobile phase composition</td>
<td>55</td>
<td>100.45</td>
<td>1.10</td>
</tr>
<tr>
<td>Percent acetonitrile</td>
<td>45</td>
<td>100.72</td>
<td>0.87</td>
</tr>
<tr>
<td>Column temperature (°C)</td>
<td>29</td>
<td>100.20</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>100.45</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Stability
The stability of drug standard solutions was tested at several storage conditions (room temperature in the dark for 24 h; autosampler conditions for 24 h and 4°C for 1 month). Stability studies indicated that the samples were stable when kept at room temperature for 24 h, in autosampler conditions for 24 h and refrigerated at 4°C for 1 month. Under all conditions tested, EPR and HCT were found to be stable.

Robustness
The robustness of the HPLC method was evaluated by changing the flow-rate, column oven temperature and acetonitrile and water phase contents of the mobile phase. The mobile phase proportions were changed from 55:45 (acetonitrile–acidic solution) to 45:55; column temperature was changed from 29 to 31°C; and the flow rate was changed from 0.95 to 1.05 mL/min. These changes had no significant effect on peak area. Low RSD values were indicative of the robustness of the method (Table III).

System suitability parameters of study are given in Table IV.

Applications of the methods
The applicability of the proposed methods was tested by the determination of drugs in their pharmaceutical preparations. The results of the HPLC method were statistically compared with those obtained by the derivative UV spectrophotometric method. The Student’s t-test and variance ratio F-test revealed no significant difference in terms of averages and SDs (Table V).

Conclusion
A first-derivative spectrophotometric method and an LC method were developed for the determination of EPR and HCT in the presence of each other. The proposed first-derivative spectrophotometric method is simple, practical, inexpensive and fast. The HPLC method has rapid and simple mobile phase and sample preparation steps, improved sensitivity and a short chromatographic run time. Both methods were validated and the acquired validation parameters indicated that the proposed method is selective, precise, accurate and robust, and hence, suitable for routine analysis and quality control of these drugs in tablets.

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References
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2. McClellan, K.J., Balfour, J.A.; Eprosartan; Drugs (1999); 55: 713.
22. Bhalotra, A., Puri, B.K.; Simultaneous first derivative spectrophotometric determination of palladium and nickel using 2-(2-thiazolylazo)-5-dimethylaminobenzoic acid as an analytical reagent; Mikrochimica Acta (2000); 134: 139.