Simultaneous Spectrophotometer Determination of Propranolol Hydrochloride and Hydrochlorothiazide in Pharmaceutical Formulations

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Three simple, accurate and economic methods, derivative spectroscopy, multicomponent and two-wavelength, have been described for the simultaneous determination of propranolol hydrochloride and hydrochlorothiazide in dosage formulations. Propranolol hydrochloride shows absorbance maxima at 289 nm and hydrochlorothiazide shows absorbance maxima at 271.2 nm in 0.1 N HCl. The methods allow rapid analysis of binary pharmaceutical formulation with accuracy. Both the drugs show linearity with absorbances in the concentration ranges employed for the method. The methods have been validated statistically and by recovery studies.

Propranolol hydrochloride (PPL) and hydrochlorothiazide (HCT) are known to have a synergistic therapeutic effect in essential hypertension. PPL is a non-selective β-blocker and HCT is a diuretic and a combination of these two is usually prescribed in the initial management of essential hypertension. Fixed combination of PPL (40 mg) and HCT (25 mg) is available in the market as tablet.

The IP and BP specify spectrophotometric determination and USP specifies HPLC method for the analysis of PPL and HCT separately. Literature survey reveals colorimetry, fluorimetric, HPLC methods for analysis of PPL separately and colorimetry, gas chromatography, fluorimetric methods for analysis of HCT alone and spectrophotometric analysis in combination with solalol. A HPLC method of simultaneous analysis of PPL-HCT tablets in combination has been described in USP. But no spectrophotometric method has been reported so far for the simultaneous estimation of PPL and HCT from dosage formulations. A successful attempt has been made to estimate them simultaneously by spectrophotometric analysis.

A Shimadzu UV/Vis spectrophotometer Model 160A was employed with spectral bandwidth of 3 nm and wavelength accuracy of 0.5 nm with automatic wavelength correction with a pair of 10 mm quartz cells. PPL (Cipla Ltd), hydrochloric acid (Ranbaxy, AR grade) and double distilled water were used in the study. Tablet formulations of combined dosage form were procured from the local market. Stock solution (100 µg/ml) of PPL and HCT were prepared by dissolving 10 mg of drug in 0.1 N HCl separately. The maximum absorbance of PPL and HCT was obtained at 289 nm and 271.2 nm, respectively. PPL and HCT show linearity with absorbances in the range 0-90 µg/ml and 0-32 µg/ml at their respective maxima, which were validated by least square method. Coefficient of correlation was found to be 0.9999 for PPL and 0.9997 for HCT. Three methods were developed for simultaneous estimation of PPL and HCT, for comparison and validation of the result. For all the three methods same six mixed standards having concentration 0, 10, 20, 30, 40 and 50 µg/ml for PPL and 30, 24, 18, 12, 6 and 0 µg/ml for HCT, respectively, were prepared by diluting appropriate volumes of the standard stock solutions. The scanning of solutions of PPL and HCT was carried out in the range of 200 nm to 400 nm (fig. 1). In multicomponent analysis three sampling wavelengths 271, 289 and 317 nm were selected for estimation of PPL and HCT. The data were fed to the instrument and then the samples were scanned; the results of which were printed out.

For estimation of one component by two-wavelength method, two wavelengths were selected where the absorbances of other component were the same. Therefore the

*For correspondence:
**Fig. 1: Overlaid spectra.**

Overlap spectra of propranolol hydrochloride (PPL) and hydrochlorothiazide (HCT) that are used in the simultaneous spectrophotometric analysis are shown.

Difference in absorbances in the mixed spectra at corresponding wavelengths and will be directly proportional to the concentration of that component. For PPL 289.0 (λ₁) and 250.4 nm (λ₂) and for HCT 271.2 (λ₁) and 302.0 nm (λ₂) were selected. All the mixed standards were then scanned at these selected wavelengths separately using quantitative mode of the instrument. The difference in the absorbance i.e., $A_{λ₁} - A_{λ₂}$ was plotted against the respective concentration to obtain the calibration curves. The sample solutions were measured at selected wavelengths and the values of difference in absorbance were extrapolated on the working standard curve to get the concentration.

The standard stock solutions of PPL and HCT were scanned from 200 nm to 400 nm and the spectra so obtained were derivetized in different orders and then recorded (fig. 2). From all the overlaid derivative spectra obtained, the wavelengths were selected in a manner such that the zero crossing of one drug should have substantial absorbance for the other drug. In the first order derivative spectra with derivative interval of 5 nm two wavelengths i.e. 293.4 nm and 288 nm were selected for estimation of PPL and HCT, respectively. The mixed standards were scanned in the spectrum mode, derivatized in first order with derivative interval of 5 nm and absorbances were measured at the selected wavelengths. These absorbances were plotted against concentration to get calibration curves. By extrapolating the value of absorbances, the concentration of the corresponding drugs in the sample was determined.

Twenty tablets were taken, their average weight was determined, and crushed to a fine powder. The powder sample equivalent to 10 mg HCT was weighted and taken in

**Fig. 2: First order derivative of overlaid spectra.**

First order derivative of the overlaid spectra of propranolol hydrochloride (PPL) and hydrochlorothiazide (HCT) that are used in the simultaneous spectrophotometric analysis are shown.

A 100 ml volumetric flask, dissolved in 0.1 N HCl and filtered through a Whatman filter paper No. 41. The filtered solution was suitably diluted to get final concentrations of 10, 15 and 25 μg/ml of HCT. Sample solutions were scanned using different methods and the results were obtained. Similar procedure was repeated three times each with different brands of the tablets [Ciplar-H (Marketed by Cipla Ltd.) and BP Norm S₉ (Marketed by Medley Ltd.)]. A preanalyzed tablet solution prepared in 0.1 N HCl containing 40 μg/ml PPL and 25 μg/ml of HCT was used for recovery studies. Different concentrations of PPL and HCT were added as shown in Table 1. The amounts of PPL and HCT recovered by scanning the solution in the respective mode for different methods were determined.

The spectral characters of PPL and HCT reveal that the absorbance maxima of PPL (289 nm) and HCT (271.2 nm) do not lie in close proximity to each other, but show, an extent of interference. Selection of the wavelengths and six mixed standards were found to give best results in multi-component mode. For the two-wavelength method the absorbance difference between two points on the spectra is directly proportional to the concentration of the component of interest independent of the interfering component is the basic principal underlying the two-wavelength method of analysis. In the third method employing derivative spectroscopy, first to fourth order derivative spectra of both the drugs were observed and first order derivative spectra was selected keeping in view the absorbances of the component at the zero crossing. The estimation of PPL and HCT was performed at 288 nm (zero crossing of PPL) 298.4 nm (zero crossing of HCT), respectively.

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TABLE 1: RESULTS OF TABLET ANALYSIS.

<table>
<thead>
<tr>
<th>Brands</th>
<th>Label claim mg/tablet</th>
<th>Amount found by method A</th>
<th>Amount found by method B</th>
<th>Amount found by method C</th>
<th>% Recovery of method A</th>
<th>% Recovery of method B</th>
<th>% Recovery of method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PPL-40</td>
<td>40.23</td>
<td>40.11</td>
<td>39.98</td>
<td>100.58</td>
<td>100.26</td>
<td>99.95</td>
</tr>
<tr>
<td></td>
<td>HCT-25</td>
<td>25.05</td>
<td>25.11</td>
<td>25.11</td>
<td>100.20</td>
<td>100.42</td>
<td>100.43</td>
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<tr>
<td>II</td>
<td>PPL-40</td>
<td>40.19</td>
<td>40.10</td>
<td>39.94</td>
<td>100.49</td>
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<td>99.84</td>
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<tr>
<td></td>
<td>HCT-25</td>
<td>25.13</td>
<td>25.11</td>
<td>25.13</td>
<td>100.50</td>
<td>100.41</td>
<td>100.51</td>
</tr>
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</table>

PPL denotes propranolol hydrochloride whereas HCT indicates hydrochlorothiazide. Where A is multicomponent method, B is two-wavelength method and C is derivative spectroscopic method. The results are the mean of six readings.

TABLE 2: STATISTICAL ANALYSIS OF RESULTS OF PPL AND HCT.

<table>
<thead>
<tr>
<th>Method</th>
<th>Brand</th>
<th>Drug</th>
<th>Tablet formulation</th>
<th>S.D.</th>
<th>C.O.V.</th>
<th>S. E.</th>
<th>Recovery studies</th>
<th>S.D.</th>
<th>C.O.V.</th>
<th>S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>I</td>
<td>PPL</td>
<td>0.9420</td>
<td>0.9365</td>
<td>0.3840</td>
<td>0.3513</td>
<td>0.3500</td>
<td>0.2028</td>
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<tr>
<td></td>
<td>II</td>
<td>PPL</td>
<td>0.9640</td>
<td>0.9592</td>
<td>0.3930</td>
<td>0.5515</td>
<td>0.5501</td>
<td>0.3184</td>
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<tr>
<td></td>
<td>I</td>
<td>HCT</td>
<td>1.0550</td>
<td>1.0520</td>
<td>0.4300</td>
<td>0.2986</td>
<td>0.2976</td>
<td>0.1723</td>
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<tr>
<td></td>
<td>II</td>
<td>HCT</td>
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<td>0.9691</td>
<td>0.3970</td>
<td>0.5075</td>
<td>0.5089</td>
<td>0.2930</td>
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<tr>
<td>B</td>
<td>I</td>
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<td>1.1750</td>
<td>1.1710</td>
<td>0.4790</td>
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<td>0.2640</td>
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<td></td>
<td>II</td>
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<td>1.2180</td>
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<td>0.5900</td>
<td>0.3411</td>
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<tr>
<td></td>
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<td>1.1620</td>
<td>0.4764</td>
<td>0.5278</td>
<td>0.5284</td>
<td>0.3047</td>
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<tr>
<td>C</td>
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<td>PPL</td>
<td>0.9544</td>
<td>0.9548</td>
<td>0.3896</td>
<td>0.2230</td>
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<td>0.1280</td>
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<tr>
<td></td>
<td>II</td>
<td>PPL</td>
<td>1.1031</td>
<td>1.0970</td>
<td>0.4502</td>
<td>0.5088</td>
<td>0.5110</td>
<td>0.2939</td>
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<tr>
<td></td>
<td>I</td>
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<td>0.9387</td>
<td>0.9401</td>
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<td>0.3103</td>
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<tr>
<td></td>
<td>II</td>
<td>HCT</td>
<td>1.1031</td>
<td>1.0970</td>
<td>0.4502</td>
<td>0.5088</td>
<td>0.5110</td>
<td>0.2939</td>
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</tbody>
</table>

PPL denotes propranolol hydrochloride whereas HCT indicates hydrochlorothiazide. Where A is multicomponent method, B is two-wavelength method and C is derivative spectroscopic method. S.D. denotes standard deviation, C.O.V. is coefficient of Variance and S. E. is standard error. The results are the mean of six readings.

The results of tablet analysis obtained by the proposed methods were validated by statistical data (Table 2) where standard deviation for PPL and HCT were found to be 1.167 and 0.9544, respectively for one brand of tablets and 1.130 and 0.9387, respectively for the other brand. Recovery study, which lies between 0.5278 and 0.2230 for the first brand and 0.5375 for the other brand indicate the reproducibility of the method. All the developed methods were found to be simple, rapid and accurate for routine simultaneous estimation of PPL and HCT in tablet formulations.

REFERENCES

Importance of the Oxidation Reaction of Sodium Metaperiodate for Spectrophotometric Assay of Tylosin

Foods and Drugs Laboratories, School of Chemistry, Andhra University, Visakhapatnam-530 003.

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A simple and sensitive spectrophotometric method by exploiting the importance of the oxidation reaction of sodium metaperiodate for the assay of tylosin has been described. This method is based on the oxidation of tylosin with excess of periodate and estimating the dialdehyde formed with 3-methyl-2-benzothiazolinone hydrazone (MBTH). The recoveries range from 99.06-100.86%.

Tylosin (TS)\textsuperscript{1-3} is a macrolide antibiotic used in veterinary medicine in the prophylaxis and treatment of various infections caused by susceptible organisms. Literature survey has revealed that little attention was paid in developing visible spectrophotometric methods\textsuperscript{4-5}. The presence of vicinal diol group in TS render it vulnerable to stoichiometric attack by periodate giving a dialdehyde. The present communication describes a method based on the oxidative coupling of the dialdehyde (from TS) with MBTH to yield a blue cationic dye\textsuperscript{6-8}.

A Milton Roy Spectronic 1201, UV/Vis spectrophotometer with 1 cm matched quartz cells was used for the absorbance measurements. All the chemicals used were of analytical grade and all the solutions were prepared with double distilled water. Aqueous solutions of sodium metaperiodate (BDH, 9.35x10\textsuperscript{-3} M), MBTH (Fluka, 8.56x10\textsuperscript{-3} M) and acetic acid (BDH, 3.49 M) were prepared.

A 1 mg/ml solution was prepared by dissolving 100 mg of pure TS in 100 ml of 0.1 M HCl and this stock solution was further diluted with distilled water to obtain the working standard solution of concentration of 50 µg/ml. An accurately weighed amount of tablet powder or measured volume of injection equivalent to 100 mg of TS was extracted with isopropanol (4x15 ml) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 100 ml of 0.1 M HCl to achieve a concentration of 1 mg/ml. The solution was further diluted with distilled water to get formulation solution of concentration 50 µg/ml.

Into a series of 25 ml calibrated tubes containing aliquots of standard TS solution (1.0-6.0 ml, 50 µg/ml solution), 1.0 ml of NaIO\textsubscript{4} and 0.5 ml of acetic acid were added. The volume was brought up to 10 ml with distilled water and kept in a boiling water bath for 10 min. After that, 1.0 ml of MBTH solution was added and heated further for 2 min. The solutions were cooled to room temperature and the volume was made up to the mark with distilled water. The absorbance