UV Analytical Method Suitability for Investigation of BCS Class 2 Biowaivers: Ibuprofen Case

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ABSTRACT
Biowaivers are scientifically justified for immediate-release oral dosage forms containing BCS Class 2 drugs. Therefore, a comparison of in vitro dissolution profiles via similarity factor factor calculation is expected. If a difference greater than 10% cannot be detected by the analytical method, then the $f_2$ similarity factor will not detect any differences between profiles. The aim of the present study was to evaluate the sensitivity of UV measurements of a Class 2 drug, ibuprofen, at the three physiological pH values of biowaiver analysis and at the different wavelengths according to USP and Ph. Eur. The slope of the calibration curve and the discriminant capacity were calculated to evaluate the sensitivity of each method. It was concluded that at 264/272 nm (identification Ph. Eur. and USP wavelengths), the analytical method is not suitable for ibuprofen biowaiver investigation, while at 220/221 nm (USP dissolution test), the UV method has adequate sensitivity.

INTRODUCTION
The Biopharmaceutics Classification System (BCS) guidances (1, 2) allow a waiver of in vivo bioequivalence studies for immediate-release oral dosage forms containing BCS Class 1 drugs (rapidly dissolving and with similar dissolution profiles to the reference product at pH values of 1.2, 4.5, and 6.8). Further discussions and subsequent publications (3, 4) recommend that biowaivers can be extended to BCS Class 2 weak acids (high solubility at pH 6.8 but not at pH 1.2 or 4.5, high permeability) if the multisource product is rapidly dissolving at pH 6.8 and its dissolution profile is similar to that of the reference at the three pH values. Ibuprofen (IBU) is a Class 2 drug (5); therefore, biowaivers for its immediate-release dosage forms are under investigation (6, 7). Besides, this NSAID is one of the most-used anti-inflammatory drugs, with a large number of different formulations available.

Dissolution profile similarity may be determined using the $f_2$ factor. When two profiles are identical, $f_2$ has a value of 100. An average difference of no more than 10% at any sample time point of the profiles may be acceptable, and this represents a similarity factor of 50. The dissolution profile of a test batch is therefore considered similar to that of the reference product if the $f_2$ value is not less than 50 (8).

The ability of the in vitro dissolution test to detect differences is of great importance for biowaiver definitions. Thus, the sensitivity of the analytical method used to measure the dissolution samples is also of great importance. The USP dissolution test for IBU immediate-release tablets uses quantification by UV spectrophotometry at the wavelength of maximum absorbance (about 221 nm), while HPLC with UV detection at 220 nm is recommended for IBU oral suspensions (9). According to USP (9) and Ph. Eur. (10), IBU is identified by UV absorption at about 264 and 272/273 nm in 0.1 N sodium hydroxide. It is known that absorbance wavelength and sensitivity of measurements can vary according to the solvent in which the analyte is dissolved. Investigation of the possibility of biowaivers for IBU was carried out by Alvarez et al. (7) using UV spectrophotometry according to Ph. Eur.

The purpose of the present study was to evaluate the sensitivity of UV measurements of a Class 2 drug, IBU, at the three physiological pH values and different wavelengths according to USP and Ph. Eur., to verify the suitability of the analytical method for biowaiver studies. The sensitivity of each method was evaluated through the slope of the calibration curve and the discriminant capacity.

MATERIALS AND METHODS
Reagents
IBU Ph. Eur. bulk drug (99.8% purity, 0.100% humidity) was purchased from Guinama (Valencia, Spain). Hydrochloric acid, glacial acetic acid, potassium chloride, sodium acetate trihydrate, sodium hydroxide, and monobasic potassium phosphate were purchased from Panreac (Barcelona, Spain). High purity deionized water was obtained from a Milli-Q purification system (Millipore, Bedford, USA). Buffer solutions of pH 1.2, 4.5, and 6.8 were prepared according to USP (9).

Equipment
The pH values of buffer solutions were measured with a Crison pH meter (model GLP 22). A UV–vis double-beam spectrophotometer (Shimadzu UV–1700 Pharmaspec) was used.

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Linearity and Sensitivity

Linearity was determined in triplicate at the three pH values according to ICH (11). Absorbance values were recorded at wavelengths of pharmacopeial requirements: 220, 221, 264, and 272 nm (9, 10). The linearity was statistically evaluated using Statgraphics Plus v.5.1 (StatPoint Technologies, Inc., Warrenton, VA). The sensitivity was evaluated by two parameters: the slope of the calibration curve and the discriminant capacity. Discriminant capacity is defined as the smallest difference of analyte concentration that can be recorded by the method for a given probability (12).

Sample Solutions

IBU has very low solubility at acidic pH. The maximum concentration of dissolved IBU obtained in pH 1.2 buffer solution was 0.02 mg/mL, and 0.03 mg/mL in pH 4.5 buffer. These values were considered the upper limit of test concentration (120% of test concentration). From these stock solutions, successive dilutions were made to obtain linearity test samples in the range of 12–120% of test concentration.

RESULTS AND DISCUSSION

Results for linearity are shown in Table 1. The linear regression method was highly significant ($p < 0.01$), and the $y$-intercept did not differ from zero for all wavelengths in all cases. For gastric pH, the slopes of the regression curves were around 0.0045 at 220/221 nm. However, at 264/272 nm the slopes were reduced 20-fold, showing the lowest calibration sensitivity obtained for all pH values and wavelengths studied. At pH 4.5, all slopes were double those of pH 1.2. At pH 6.8, the slope of the regression curve at 220/221 nm was almost double those obtained at 264/272 nm.

The differences among the slopes are clearly shown in Figure 1. The greatest sensitivities were obtained at pH 6.8, followed by pH 1.2 and pH 4.5. Although the y-intercept did not differ from zero for all wavelengths in all cases. For gastric pH, the slope of the regression curve at 220/221 nm was almost double those obtained at 264/272 nm. At pH 4.5, all slopes were double those obtained at pH 1.2 and pH 6.8. In all cases, the greatest sensitivity was obtained at pH 2.2, followed by pH 4.5 and pH 6.8. In alkaline pH, IBU is highly soluble. The maximum concentration of IBU at pH 6.8 was 0.5 mg/mL. Twelve levels of dilution were evaluated, between 0.6% and 120% of test concentration (0.42 mg/mL).

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### Table 1. Linearity of IBU Solutions at Four Wavelengths in Physiological pH

<table>
<thead>
<tr>
<th>pH</th>
<th>% test concentration (mg/mL)</th>
<th>$\lambda$ (nm)</th>
<th>Slope (SE)</th>
<th>$y$-intercept (SE)</th>
<th>t-test, $y = 0$</th>
<th>$r^2$</th>
<th>Residual sum of squares</th>
<th>ANOVA linear model</th>
<th>F-test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>12–120 (0.002–0.02)</td>
<td>220</td>
<td>0.00455 (0.00017)</td>
<td>0.00144 (0.01262)</td>
<td>0.9104</td>
<td>0.9774</td>
<td>0.01262</td>
<td>690.87 (p &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>221</td>
<td>0.00452 (0.00017)</td>
<td>0.00045 (0.01249)</td>
<td>0.9715</td>
<td>0.9775</td>
<td>0.01236</td>
<td>695.14 (p &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>264</td>
<td>0.00022 (0.0002)</td>
<td>0.00328 (0.00170)</td>
<td>0.0722</td>
<td>0.8513</td>
<td>0.00023</td>
<td>91.63 (p &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>272</td>
<td>0.00018 (0.0002)</td>
<td>0.00209 (0.00134)</td>
<td>0.1378</td>
<td>0.8577</td>
<td>0.00014</td>
<td>96.44 (p &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>6–120 (0.0015–0.03)</td>
<td>220</td>
<td>0.00959 (0.00038)</td>
<td>0.00732 (0.24961)</td>
<td>0.7720</td>
<td>0.9662</td>
<td>0.11597</td>
<td>628.89 (p &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>221</td>
<td>0.00972 (0.00037)</td>
<td>0.00642 (0.02428)</td>
<td>0.7938</td>
<td>0.9687</td>
<td>0.10975</td>
<td>681.66 (p &lt; 0.01)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>264</td>
<td>0.00043 (0.0002)</td>
<td>0.00105 (0.00122)</td>
<td>0.4015</td>
<td>0.9607</td>
<td>0.00028</td>
<td>537.71 (p &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>272</td>
<td>0.00035 (0.00001)</td>
<td>0.00066 (0.00089)</td>
<td>0.4682</td>
<td>0.9673</td>
<td>0.00015</td>
<td>649.79 (p &lt; 0.01)</td>
<td></td>
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<tr>
<td>6.8</td>
<td>6–120 (0.0025–0.05)</td>
<td>220</td>
<td>0.16797 (0.00226)</td>
<td>0.02105 (0.01279)</td>
<td>0.1162</td>
<td>0.9966</td>
<td>0.02622</td>
<td>5507.64 (p &lt; 0.01)</td>
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<tr>
<td></td>
<td></td>
<td>221</td>
<td>0.17176 (0.0022)</td>
<td>0.02102 (0.01268)</td>
<td>0.1138</td>
<td>0.9968</td>
<td>0.02575</td>
<td>5863.86 (p &lt; 0.01)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>264</td>
<td>0.00745 (0.0006)</td>
<td>0.00027 (0.00306)</td>
<td>0.9305</td>
<td>0.9979</td>
<td>0.00680</td>
<td>16398.25 (p &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>272</td>
<td>0.00620 (0.0005)</td>
<td>0.00010 (0.00253)</td>
<td>0.9682</td>
<td>0.9980</td>
<td>0.00464</td>
<td>16648.09 (p &lt; 0.01)</td>
<td></td>
</tr>
</tbody>
</table>
Discriminant capacity values are shown in Figure 2. For all pH levels studied, the smallest difference between analyte concentrations was less than 10% only at 220/221 nm. This level of discrimination was sufficient to detect the minimum difference between profiles needed to obtain a similarity factor of 50. The UV method at 264 nm for pH 1.2 was not able to detect such 10% differences, because the discriminant capacity was roughly 24%.

CONCLUSIONS
The highest calibration sensitivity is obtained at 220/221 nm for IBU UV measurements at the three physiological pH values. At the Ph. Eur. identification wavelength (264 nm), the UV method is not sensitive enough to detect the 10% difference between IBU concentrations required for $f_2$.

According to the results obtained in this work, the measurements for IBU biowaiver investigations might be carried out at 220/221 nm to obtain suitable sensitivity for discrimination between dissolution profiles.

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