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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 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 221nm), 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 concentrations were considered the upper range value (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 (0.017 mg/mL) at pH 1.2, with eight concentration levels. In the case of pH 4.5 buffer, the range was 6–120% of test concentration (0.025 mg/mL), with six concentration levels.

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).

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 obtained at pH 1.2. A 20-fold reduction in slope value was also produced at 264/272 nm compared with that at 220/221 nm. At jejunum pH (6.8), the slope of the method almost duplicated the slope obtained at pH 4.5, with a large difference between the slopes at 220/221 nm and those at 264/272 nm.

The differences among the slopes are clearly shown in Figure 1. The greatest sensitivities were obtained, in all cases, with UV measurements at 220/221 nm. At those wavelengths, the range of concentrations was reduced at pH 6.8 to conserve linearity (0.0025–0.05 mg/mL). This is a special feature of this UV method, which makes it useful for dissolution test of IBU oral suspensions, which have lower doses. A very low sensitivity method was obtained for quantification at the IBU determination wavelength specified in *Ph. Eur.* (264 nm), especially at acidic pH.

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

pH	% test concentration (mg/mL)	λ (nm)	Slope (SE)	*y*-intercept (SE)	*t*-test, $y = 0$ *p*-value	r^2	Residual sum of squares	ANOVA linear model *F*-test (*p*-value)
1.2	12–120 (0.002–0.02)	220	0.00455 (0.00017)	0.00144 (0.01262)	0.9104	0.9774	0.01262	690.87 ($p < 0.01$)
		221	0.00452 (0.00017)	0.00045 (0.01249)	0.9715	0.9775	0.01236	695.14 ($p < 0.01$)
		264	0.00022 (0.00002)	0.00328 (0.00170)	0.0722	0.8513	0.00023	91.63 ($p < 0.01$)
		272	0.00018 (0.00002)	0.00209 (0.00134)	0.1378	0.8577	0.00014	96.44 ($p < 0.01$)
4.5	6–120 (0.0015–0.03)	220	0.00959 (0.00038)	0.00732 (0.24961)	0.7720	0.9662	0.11597	628.89 ($p < 0.01$)
		221	0.00972 (0.00037)	0.00642 (0.02428)	0.7938	0.9687	0.10975	681.66 ($p < 0.01$)
		264	0.00043 (0.00002)	0.00105(0.00122)	0.4015	0.9607	0.00028	537.71 ($p < 0.01$)
		272	0.00035 (0.00001)	0.00066 (0.00089)	0.4682	0.9673	0.00015	649.79 ($p < 0.01$)
6.8	6–120 (0.0025–0.05)	220	0.16797 (0.00226)	0.02105 (0.01279)	0.1162	0.9966	0.02622	5507.64 ($p < 0.01$)
		221	0.17176 (0.0022)	0.02102 (0.01268)	0.1138	0.9968	0.02575	5863.86 ($p < 0.01$)
	0.6–120 (0.0025–0.5)	264	0.00745 (0.00006)	0.00027 (0.00306)	0.9305	0.9979	0.00680	16398.25 ($p < 0.01$)
		272	0.00620 (0.00005)	0.00010 (0.00253)	0.9682	0.9980	0.00464	16648.09 ($p < 0.01$)

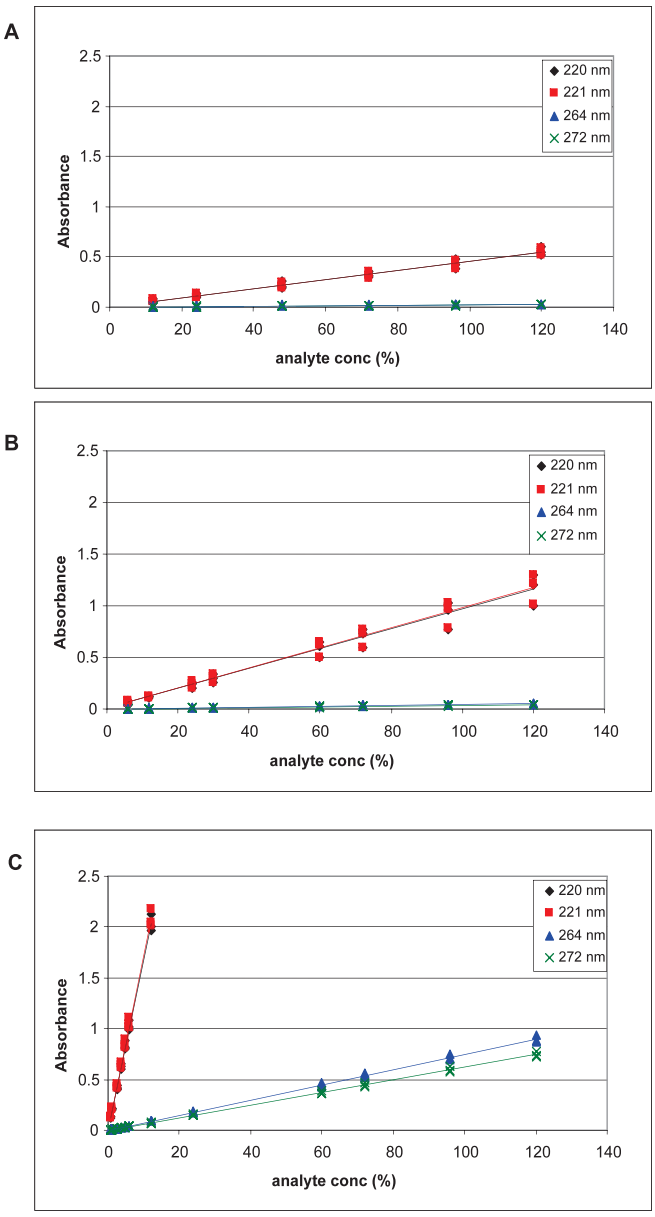

Figure 1. Sensitivity of the UV quantification method at (A) pH 1.2, (B) pH 4.5, and (C) pH 6.8 at various measurement wavelengths.

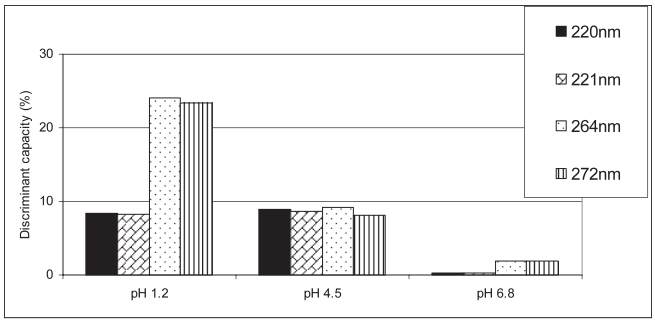

Figure 2. Discriminant capacity.

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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