Ultraviolet (UV) Spectrophotometric Analysis of Ketoprofen in Tablets. Statistical Validation of Proposed Method

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Abstract: The aim was to exactly quantify the amount of pure ketoprofen of a pharmaceutical by a new, developed ultraviolet (UV) spectrophotometric method. The maximum absorption wavelength was determined to be at λ = 254 nm for a ketoprofen alcoholic standard solution of 1.4 µg/mL. The applied method was statistically validated. The amount of pure ketoprofen assigned on the pharmaceutical tablet was found to be 146.326 mg ketoprofen/tablet. This obtained value was very close to the official declared content of active substance (150 mg pure ketoprofen/tablet), with an average percentage deviation of 2.45 %, below the maximum value (± 5 %).

Keywords: ultraviolet (UV) spectrophotometric method; to exactly quantify; the amount; pure ketoprofen; pharmaceutical tablet; the applied method; official declared content; active substance; average percentage deviation.

Chemical reagents and equipment. **Ketoprofen stock solution 1000 µg/mL (0.1%) prepared from ketoprofen crystalline pure standard powder (Merck ®), dissolved in absolute methanol as solvent; ketoprofen working solution 200 µg/mL, directly obtained by dilution (1:5) with absolute methanol from the stock solution 1000 µg/mL; a series of nine pure standard ketoprofen solutions with concentrations that range between 2.0 µg/mL – 80 µg/m, obtained from the working 200 µg/mL ketoprofen solution; analyzed sample solution obtained in absolute methanol from the tablets of a pharmaceutical; CECIL CE 3200 UV-VIS Spectrophotometer provided with four quartz tubs.*The design of UV absorption spectrum and determination of the maximum absorption wavelength of a pure ketoprofen standard solution:* the absorption spectrum was plotted and maximum absorbance wavelength was determined to be at λ = 254 nm, for a ketoprofen pure standard solution of Ce = 1.4 µg/mL = 0.00014 % = 1,4 .10 $^{-4}$ %. Maximum absorbance value corresponding to the wavelength λ = 254 nm was A = 0.1266. Specific absorbance (specific absorptivity coefficient) was calculated: a = A / Ce = 0.1266 /0.00014 = 904.2857 . Specific absorbance was: a = 904.2857.**

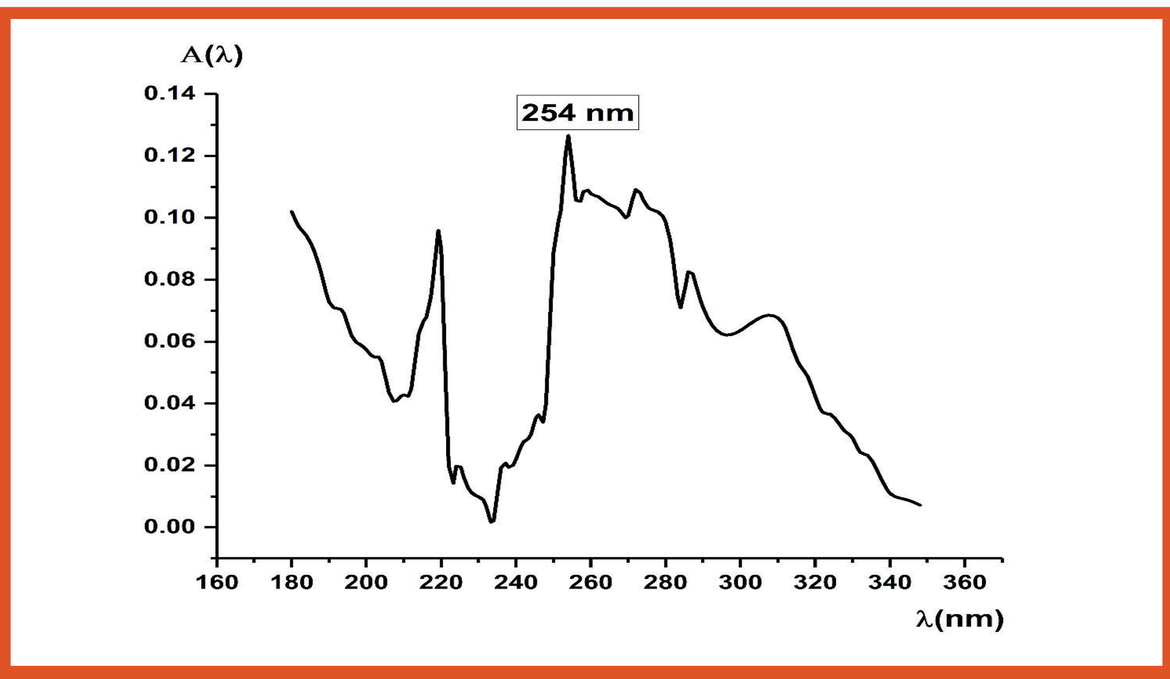
Calculation procedure of Ketoprofen studied sample **has consisted in the following stages according to Ketoprofen :**

1. Calculation of sample solution concentration C_S (µg/mL) from the regression line described in Figure 1: from the calibration graph y + 0.005 x + 0,1196, whereas y = A_S= 0.398 = sample solution absorbance has resulted x = sample solution concentration C_S (µg/mL) = (0.398 – 0.1196) / 0.005 = 55.68 (µg/mL). Thus, C_S (µg/mL) = 55.68 (µg/mL).

2. Quantitative analysis of pure Ketoprofen from the pharmaceutical tablets was assigned as follows: the amount of pure Ketoprofen from the volume V_T = 10 mL graduated glass test tube containing the sample solution was found to be X = C_S (µg/mL) x V_T = 55.68 x 10 = 556.8 µg. So, X = 556.8 µg of pure Ketoprofen from V_T = 10 mL graduated glass test tube.

3. The amount of pure Ketoprofen in the initial volume V_S = 50 mL of sample solution was: X_1 = (V_S . X) / v_S = (50 . 556.8) / 1.9 = 14652.63 µg. X_1 = 14652.63 µg pure Ketoprofen from V_S = 50 mL sample solution.

4. The amount of pure Ketoprofen on pharmaceutical tablet was found to be: Y = (m_T .X_1) / a = (0.5113 .146523.63) / 0.0512 = 146325.97 µg pure Ketoprofen / pharmaceutical = 146.326 mg pure Ketoprofen / pharmaceutical tablet. Thus, Y = 146.326 mg pure Ketoprofen / pharmaceutical. tablet was the final result. The official declared amount of pure Ketoprofen on pharmaceutical tablet, by manufacturing company was 150 mg ketoprofen / tablet. The calculated value assigned to 146.326 mg pure Ketoprofen / pharmaceutical tablet. has represented 97.55% from the official declared content of pure Ketoprofen, set out by the producing pharmaceutical company. The mean percentage deviation was only 2.45 % from the official declared amount (150 mg) of active substance. All the statistical parameters of the regression line were calculated in Microsoft Excel 2019 and described in Table 1 and Table 2. (Data → Data Analysis → Regression → Regression Statistics).



Absorption UV Spectrum of Ketoprofen

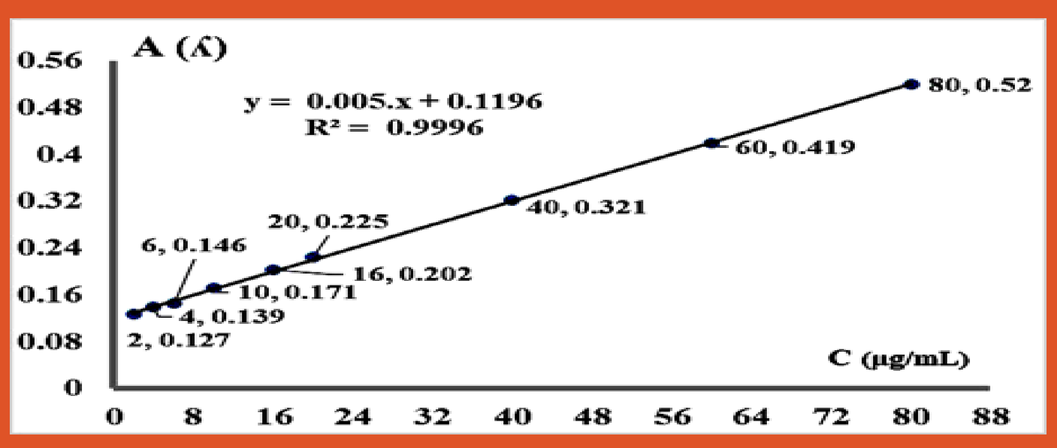
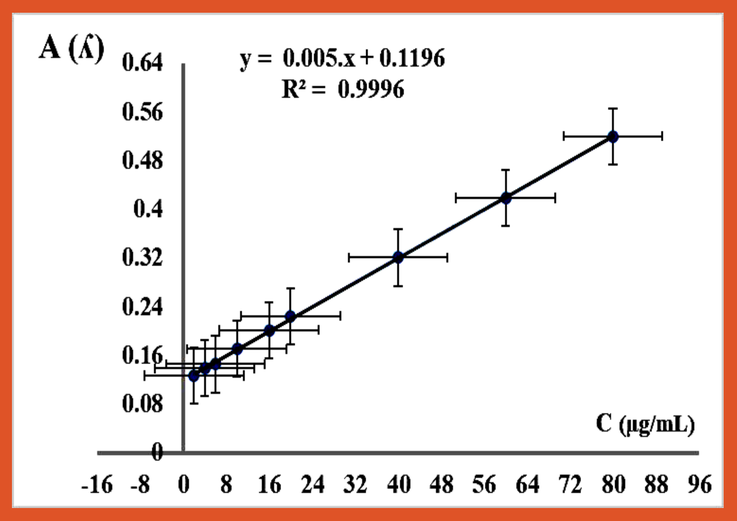


Figure 1. Calibration graph designed for Ketoprofen standard solutions (2.0 µg/mL – 80.0 µg/mL) at λ = 254 nm, against absolute methanol as a blank)



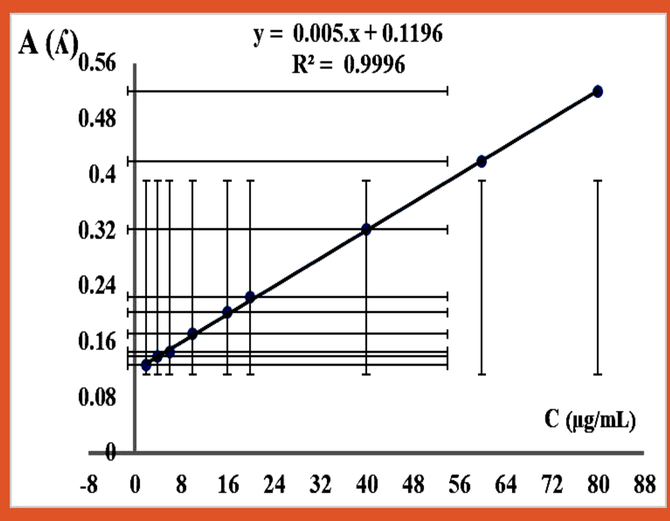


Figure 2. (a) Graphical errors amount: Standard Error (SE) disclosure for the calibration graph of ketoprofen pure standard solutions (2.0 µg/mL- 80.0 µg/mL); (b) Description of Standard Deviation (s) (SD) of the regression line for ketoprofen pure standard solutions (2.0 µg/mL- 80.0 µg/mL); Statistically meaning, both, Standard Errors (SE) and Standard Deviations (SD) were found within the normal range of required values , as shown in Table 2:

Table 1. Statistical Values of Linear Regression Parameters. and Descriptive Statistics

Regression statistics	Statistical values	Ae (λ)	Ce (µg/mL)
Multiple R (Correlation coefficient)	0.999816	0.127	2.0
Mean	0.252222	0.139	4.0
Median	0.202	0.146	6.0
Confidence Level (95.0 %) of Absorbances	0.106611	0.171	10.0
R square, R^2 (Linear Regression coefficient)	0.999631	0.202	16.0
Sample variance	0.019237	0.225	20.0
Adjusted R, Square R^2	0.999579	0.321	40.0
Standard Deviation (SD)	0.138696	0.419	60.0
Standard Error (SE) of the Regression Line	0.002847	0.520	80.0
Standard Error of Measured Absorbances	0.046232	Count:	9.0

Table 2/ Concentration of the sample and the amounts of Pure Ketoprofen calculated Pharmaceutical tablet

Sample Absorbance (A_S)	Sample CS (µg/mL)	mg pure Ketoprofen/ Tablet
0.398	55.68	146.326
Covariance P = 3.408790	Percentage Content: 97.55%	Mean Percentage Deviation: 2.45%

Conclusions. The method applied for Ultraviolet (UV) spectrophotometric analysis of pure Ketoprofen form the tablets of a pharmaceutical presented a very good linearity over the entire chosen concentration range 2.00 µg/mL- 80.0 µg/mL. Regression coefficient was R^2 = 0.999631 $R^2 \geq 0.9990$ and correlation coefficient R = 0.999816. R > 0.9990 were fit perfectly fit within the normal range of values. Standard error of the regression line SE = 0.002847 and the Detection LD and Quantitation limits LQ were reported to be within the normal values LD = 1.7082 µg/mL and LQ = 5.694 µg/mL. Covariance coefficient was 3.408790 and Sample variance was assigned to 0.019237. Both parameters had very small values, below the maximum allowed limit (≤ 5%). The amount of pure Ketoprofen on pharmaceutical tablet was found to be 146.326, mg/ tablet of a pharmaceutical very close to the declared content of active substance (150 mg), with as mean percentage deviation of only 2.45% compared to the official declared value. This percentage was far below the maximum percentage deviation allowed deviation to the declared active substance content (± 5%) provided by Romanian Pharmacopeia and by the European and International Pharmacopoeias. So that the studied pharmaceutical product fal perfectly within the limits of normal values provided by these pharmacopoeias.