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

Cristian-Catalin Gavat *

University of Medicine and Pharmacy "Grigore T. Popa", Biomedical Sciences Department, Faculty of Medical Bioengineering, 16 Universitatii Street, Iasi 700115, Romania; ccgavat70@yahoo.com; Tel.: +40-743-782544

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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 $\lambda = 254$ 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

1. Introduction

Ketoprofen is an effective anti-inflammatory agent, part of non-steroidal anti-inflammatory drugs (NSAIDs) category. From a structural point of view, it is included in carboxylic and heterocyclic acetic acids class. It is a derivative of propionic acid (an aryl propionic acid), closely related to Ibuprofen and Flurbiprofen [1,2]. Ketoprofen has a strong anti-inflammatory action, comparable to Indomethacin, as well as important antipyretic and analgesic effects. It equally inhibits both Cyclooxygenase isoforms (COX-1 and COX-2) as well as lipoxygenase. By inhibiting both Cyclooxygenase COX-1 and COX-2, there is a considerable decrease in the synthesis of precursors of prostaglandins and thromboxane [1–9]. The pronounced decrease in prostaglandin synthesis, by effectively blocking prostaglandin-synthase activity, is directly responsible for the therapeutic effects of another anti-inflammatory drug, Ibuprofen. In addition to this therapeutic effect manifested by Ibuprofen, Ketoprofen also causes a important decrease in Thromboxane—A2 synthesis by blocking thromboxane-synthase enzyme, thereby effectively inhibiting platelet aggregation [1–9]. A 2013 systematic review indicated that "The efficacy of orally administered Ketoprofen in relieving moderate-severe pain and improving functional status and general conditions was significantly better than that of Ibuprofen and/or Diclofenac" [4].The anti-inflammatory effects of Ketoprofen are believed to be due to Cyclooxygenase (COX-2) effective inhibition, which is an enzyme involved in prostaglandin synthesis via Arachidonic acid pathway. This conducts to pronounced decreased levels of prostaglandins that mediate pain, fever and inflammation. Ketoprofen, as a non-specific Cyclooxygenase inhibitor and inhibitor of COX-1 it is thought to confer some of its side effects such as Gastro Intestinal (G.I.) upset (dyspepsia, diarrhea, abdominal pain, constipation, flatulence) and ulceration. Ketoprofen it is thought to have anti-bradykinin activity as well as lysosomal membrane-stabilizing action. Antipyretic effects may be due to direct action on

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the hypothalamus, resulting in an increased peripheral blood flow, vasodilatation and subsequent heat dissipation [1–9]. It is highly recommended in the treatment of mild to moderate pains caused by inflammations. Ketoprofen is one of the most important drugs successfully administered for the treatment of rheumatoid polyarthritis, spondylitis, arthrosis, disc diseases, acute arthritis, gut attacks [3–7]. It is very effective for the symptomatic treatment of acute and chronic rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, primary dysmenorrhea and mild to moderate pain associated with musculotendinous trauma (sprains and strains), postoperative pain (including dental surgery) and postpartum pain [3–8]. A very important and particular aspect in pharmaceutical chemistry and also in chemical and biochemical laboratory has always been the quantitative spectrophotometric analysis of Ketoprofen from various studied samples such as different and various brand of pharmaceuticals, as well as from biological fluids, in chronic and acute intoxications [5,10]. *The aim of this research* was to exactly quantify the amount of pure ketoprofen of a pharmaceutical by a new, developed ultraviolet (UV) spectrophotometric method. This experimental procedure has been designed, created, developed, proposed to be statistically validated [11–14] and subsequently applied successfully for quantitative analysis of Ketoprofen from various samples.

2. Materials and Methods

2.1. Method Description

Pure ketoprofen from the tablets of a chosen pharmaceutical was quantitatively analyzed by UV spectrophotometry to the maximum absorption wavelength of $\lambda_{max.}$ = 254 nm, in relation to absolute methanol as a reagent blank.

2.2. 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.

2.3. 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.

(a) Working procedure: preparation of ketoprofen standard solutions (2 μg/mL–80 μg/mL) and spectrophotometer calibration to λ = 254 nm, against absolute methyl alcohol as a blank: 0.05 g of ketoprofen pure crystalline standard powder supplied by Merck® was exactly weighed and quantitatively brought with a necessary volume of absolute methyl alcohol into a Berzelius beaker, under vigorous stirring until complete dissolution. The alcoholic obtained solution was completely transferred to a V = 50 mL volumetric flask under stirring conditions and then made up to the mark with absolute methanol. Thus, a stock ketoprofen solution 1000 μg/mL (0.1%) was synthetized. A working solution 200 μg/mL was prepared directly from this stock solution by accurately measuring 10 mL solution 1000 μg/mL and transferring this volume into another V_1 = 50 mL volumetric flask, which was made up to the mark

with absolute methyl alcohol. A series of nine volumes taken from this working obtained solution 200 μg/mL were accurately measured and quantitatively added into v = 10 mL different graduated glass tubes under stirring. Each of nine volumes was brought to 10 mL with absolute methyl alcohol. The mean absorbances of nine prepared ketoprofen standard solutions were exactly measured, according to their concentrations, in relation to absolute methanol as a blank, at λ + 254 nm. These values were described in Figure 1 and Table 1.

(b) Statistical study of the method linearity. Calculation methods of Detection limit LD and Quantitation limit LQ. Statistical analysis of some important parameters of the linear regression. According to the mean absorbances values of nine standard Ketoprofen solutions obtained and rendered in Figure 1 and Table 1, the calibration graph was plotted and described in (Figure 1). A statistical study was undertaken, from the point of view of the standard error analysis of the regression line (SE) and the standard deviations (SD). The suggestive graphics and statistical obtained values were described in Figure 2 and Table 1. The Detection Limit LD, as the smallest amount of analyte that could be detected in a known sample compared to a blank under established experimental conditions, was evaluated using formula: LD = 3 x Standard Error (SE)/slope (1). Quantitation limit LQ, which was described by the lowest analyte concentration in a sample that could be quantified with a statistically acceptable precision and accuracy under the same experimental conditions was calculated as follows: LQ = 10 x Standard Error (SE)/slope (2) [11,12,14]. The standard deviation (SD) [11–14] is a measure of how widely the values of a konwn sample are dispersed from the average value (the mean). Standard deviation was calculated using "STDEV" in Microsoft Excel (Table 1). Standard error of the regression line (SE) is the average distance that the observed values fall from the regression line. The standard error of the linear regression (SE) provides the absolute measure of the typical distance that the data points fall from the regression line [11–13]. The population covariance, analyzed with "COVARIANCE P" function in Microsoft Excel, represents the average of the products of deviations for each data point pair in two data sets. Covariance is always used to determine the direct relationship between two data sets [11–14].

(c) Working procedure: obtaining the sample ketoprofen alcoholic solution: sample ketoprofen solution was prepared by exactly weighing a = 0,0512 g of pharmaceutical triturated powder which was completely dissolved and quantitatively brought into a separated volumetric flask of volume V_S = 50 mL with absolute methyl alcohol, diluted to the mark. From the resulting ketoprofen sample solution, a volume V_S = 1.9 mL was accurately measured and brought into a clean graduated glass tube of volume V_T = 10 mL, which was made up to the mark with absolute methanol. The average mas of a pharmaceutical tablet containing ketoprofen as active substance was m_T = 0.5113 g = 511.3 milligrams. According to the producing company, a pharmaceutical tablet contained 150 milligrams (mg) of pure ketoprofen as active substance. Thus, the sample ketoprofen solution was prepared and the mean absorbance A_S = 0.398 was determined at λ = 254 nm against absolute methyl alcohol as a reagent blank in the same conditions as for prepared standard solutions...Measured mean absorbance of the sample and related ketoprofen sample solution calculation values was rendered in Table 2. Depending on the A_S sample absorbance, pure Ketoprofen concentration FROM the sample solution was determined to λ = 254 nm.

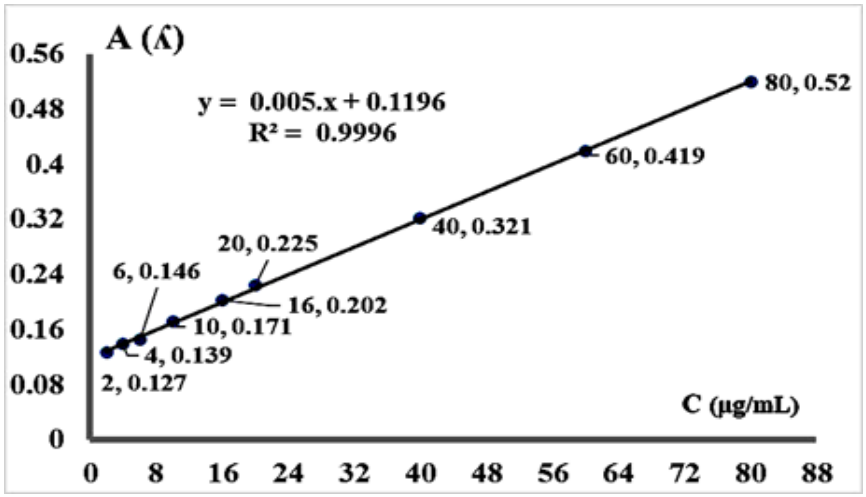
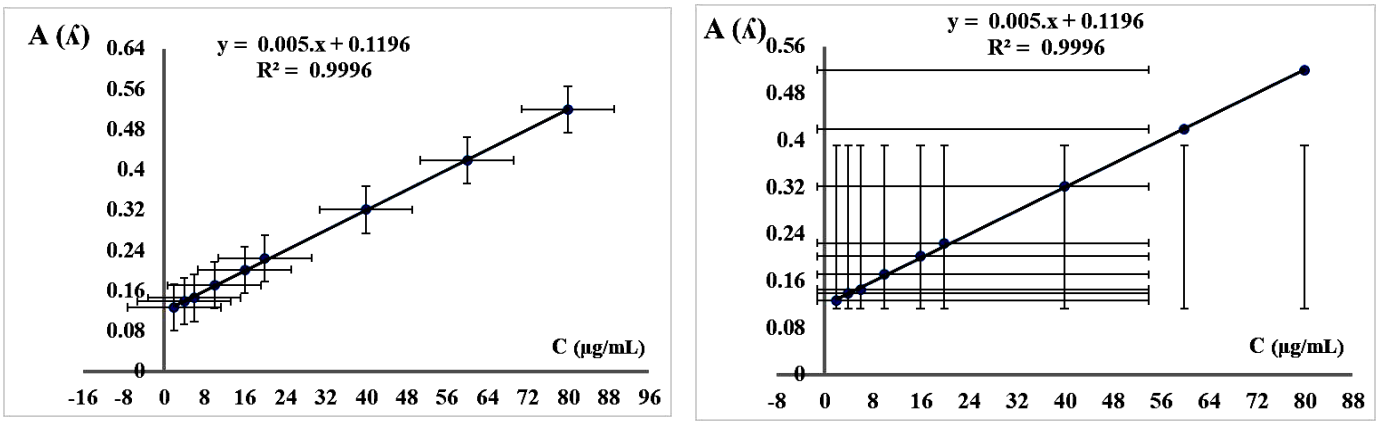


Figure 1. Calibration graph designed for Ketoprofen standard solutions (2.0 µg/mL–80.0 µg/mL) at λ = 254 nm, against absolute methanol as a blank The coordinates of each of nine points located on the regression line were highlighted.



(**a**) (**b**)

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%**

3. Results and Discussion

3.1. Heading

Calculation procedure of Ketoprofen studied sample has consisted in the following stage according to Ketoprofen sample preparation stages, described in Section 2.3 (c) paragraph.

3.1.1. Heading

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).

3.1.2. Heading

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) × V_T = 55.68 × 10 = 556.8 μg. So, X = 556.8 μg of pure Ketoprofen from V_T = 10 mL graduated glass test tube. 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 Vs = 50 mL sample solution. 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 Microsofdt Excel 2019 and described in Table 3. (Data → Data Analysis → Regression → Regression Statistics).

3.2. Statistical Study of the Method Linearity. Calculation methods of Detection limit LD and Quantitation limit LQ. Statistical analysis of some important parameters of the regression line

According to Formula (1) the Detection limit was LD = 1.7082 μg/mL and Quantitation limit was LQ = 5.694 μg/mL, both parameters fit within the normal range of values.

4. 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 fall perfectly within the limits of normal values provided by these pharmacopoeias.

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Conflicts of Interest: The author declare no conflict of interest.

References

1. Haţieganu, E.; Dumitrescu, D.; Stecoza, C. *Pharmaceutical Chemistry*, 2nd ed.; Medical Publishing House: Bucharest, Romania, 2010; Volume 1, pp. 592–594.
2. Xu, J.; Chen, W.; Wu, L.; Chang, A.C. Adsorption and Degradation of Ketoprofen in Soils. *J. Environ. Qual.* **2009**, *38*, 1177–1182. https://doi.org/10.2134/jeq2008.0347.
3. Ketoprofen | $C_{16}H_{14}O_3$-PubChem (nih.gov). Available Online: https://pubchem.ncbi.nlm.nih.gov/compound/ketoprofen#section=top. (accessed on 10 March 2023).
4. Sarzi-Puttini, P.; Atzeni, F.; Lanata, F.; Bagnasco, M. Efficacy of ketoprofen vs. ibuprofen and diclofenac: A systematic review of the literature and meta-analysis. *Clin. Exp. Rheumatol.* **2013**, *31*, 731–738.
5. DRUGBANK Online. Avalaible Online: https://go.drugbank.com/drugs/DB01009. (accessed on 11 March 2023).
6. Kantor, T.G. Ketoprofen: A review of its pharmacologic and clinical properties. *Pharmacotherapy* **1986**, *6*, 93–103. https://doi.org/10.1002/j.1875-9114.1986.tb03459.x.
7. Fokunang, C.N.; Fokunang, E.T.; Frederick, K.; Ngameni, B.; Ngadjui, B. Overview of non-steroidal anti-inflammatory drugs (nsaids) in resource limited countries. *MOJ Toxicol.* **2018,** *4*, 5–13. https://doi.org/10.15406/mojt.2018.04.00081.
8. Fischer János; Robin, G.C. *Analogue-Based Drug Discovery*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2006; pp. 520.
9. Mazières, B.; Rouanet, S.; Guillon, Y; Scarsi, C.; Reiner, V. Topical ketoprofen patch in the treatment of tendinitis: A randomized, double blind, placebo controlled study. *J. Rheumatol.* **2005**, *32*, 1563–1570.
10. Hunter, L.J.; Wood, D.M.; Dargan, P.I. The patterns of toxicity and management of acute nonsteroidal anti-inflammatory drug (NSAID) overdose. *Open Access Emerg. Med.* **2011**, *3*, 39–48. https://doi.org/10.2147/OAEM.S22795.
11. *Romanian Pharmacopoeia*, 10th Ed.; Medical Publishing House: Bucharest, Romania, 1993; pp. 977–1293.
12. Donici, E. Development and validation of spectrophotometric method in ultraviolet and visible of assay of fluocinolone acetonide from a combined ointment: Experimental study. *MJHS* **2017**, *13*, 53–58.
13. John H. McB., Miller. Validation of Pharmacopoeial Methods. In *Methods Validation in Pharmaceutical Analysis. A Guide to Best Practice,* Ermer, J., Nethercote, P.W., (Eds.); Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2005; pp. 301–335.
14. *European Pharmacopoeia*, 10th Ed.; European Directorate for the Quality of Medicine & Healthcare (edQm), Council of Europe: Strasbourg, France, 2019; Volume I, pp. 11–46.

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