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Method Development for Visible Spectrophotometric Analysis of Ibuprofen in Pharmaceuticals

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***Abstract*— Ibuprofen is a prominent member of the group of non-steroidal anti-inflammatory drugs (NSAIDs), with good anti-inflammatory action, a very effective analgesic, with increased antipyretic effect. The aim of this research was to exactly quantify pure Ibuprofen content in tablets of a pharmaceutical, by a spectrophotometric analysis method in the Visible range. Ibuprofen was quantitatively converted to a bright orange dye with a yellowish shade, by a color reaction with alpha-naphthylamine in the presence of sodium nitrite, in an absolute ethanol medium. Following the analysis, it was found 397.952 milligrams of pure Ibuprofen content / film-coated tablet of the pharmaceutical product. This value was very close to Ibuprofen content declared by the pharmaceutical manufacturer (400 milligrams), with a mean deviation of only 0.512 percent from the officially declared amount of active substance. The value found fits perfectly within the normal limits provided by the European and International Pharmacopoeias standards, taken over by the Romanian Pharmacopoeia, 10th Edition. The spectrophotometric analysis method was then successfully subjected to statistical analysis.**

Keywords*— *Ibuprofen; spectrophotometric analysis; anti-inflammatory action; film-coated tablet; statistical analysis

I. INTRODUCTION

Ibuprofen, a propionic acid derivative, is a non-steroidal anti-inflammatory drug part of the NSAIDs family. It is a very effective analgesic with a good antipyretic effect. It also has a strong anti-inflammatory action. Ibuprofen was introduced on the marketplace in 1969, as a better alternative to Aspirin. Gastric discomfort, nausea, and vomiting effects, though less intense than in the case of aspirin or indomethacin, are still the most common side effects [1-4]. It is the most commonly used and most frequently prescribed NSAID. It is a non-selective inhibitor of cyclo-oxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2). Although its anti-inflammatory properties may be weaker than those of some other NSAIDs like Piroxicam, the Coxib family, Indomethacin, Ketorolac, it has a prominent, strong analgesic action and an increased antipyretic role [1-5].

Spectrophotometric analysis of Ibuprofen in the Visible range (VIS) has always been an important concern in pharmaceutical research. This paper, has developed, improved, and optimized a spectrophotometric method, based on literature data [6], which has been successfully applied for quantitative analysis of Ibuprofen from pharmaceuticals and subjected to statistical analysis [7]. The standards and rules of the European and International Pharmacopoeias, taken over by the Romanian Pharmacopeia X-th Edition, attests that for an officially declared content of active substance on pharmaceuticals, of 100 mg and above 100 mg, the maximum allowed percentage deviation (percentage error) is no more than 5% [8].

II. MATERIALS AND METHODS

A. Materials

The equipment and materials used for this study consisted of a Spectrophotometer UV-Visible CE 1021, CECIL® Bandwidth: 8 nm and 1 cm glass cuvettes, with Deuterium lamp disconnect key (on model CE1021) used for performing the measurements; digital analytical balance with electronic display Kern ABS / ABJ with 4 decimals; crystalline extremely pure standard of ibuprofen powder, supplied by Sigma-Aldrich ®; a stock solution of ibuprofen, 1000 µg/mL (0.1 g %), prepared from the crystalline pure standard; a standard working solution 150 µg/mL obtained directly from the stock solution Other solutions used consisted of alpha-naphthylamine 0.04% and a sodium nitrite $NaNO_2$ 4% solution. Ibuprofen Grindex® film-coated tablets,

B. Methods

The wavelength corresponding to the maximum absorption of the bright orange azo-dye obtained with a yellowish shade, the absorption spectrum shown in Fig. 1 was drawn for a separate standard solution of Ibuprofen, 8 µg/mL (0.0008%), which was prepared directly from the standard working

solution, 150 µg/mL (0.015%). The maximum absorption wavelength was found to be at λ max. = 458 nm (Fig. 1).

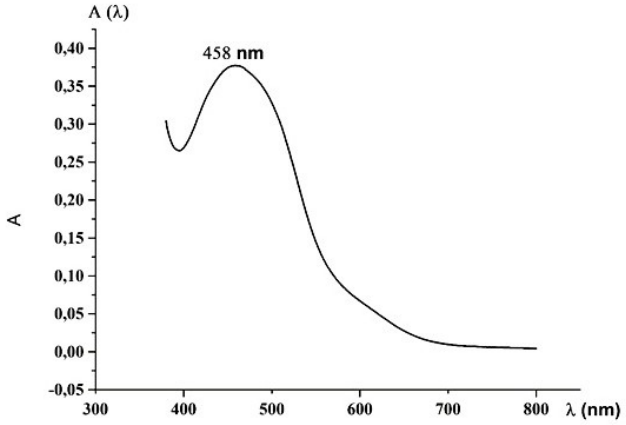

Fig. 1 Absorption spectrum of a bright orange dye with a yellowish shade obtained from ibuprofen

Specific absorptivity or specific extinction represented the absorbance (as a measure of the absorption of the selected electromagnetic radiation) of a solution layer with a thickness of 1 cm and a concentration of 1% (g / 100 mL) It was calculated and the result obtained was shown below.

Specific absorptivity:

$A^{1\%}_{1cm} = A / C_S$ (g /100 mL) (1), $C_S = 8$ µg / mL = 0.0008 g / 100 mL = standard solution concentration

A = mean measured absorbance = 0.3772

$A^{1\%}_{1cm}$ = specific absorptivity

By replacing these values in relation (1), it was obtained: $A^{1\%}_{1cm} = 0.3772 / 0.0008 = 471.50$. Thus, $A^{1\%}_{1cm} = 471.50$.

Analysis method description. Ibuprofen, 2- (4-isobutyl-phenyl) propanoic acid, a non-steroidal anti-inflammatory drug derived from propionic acid, present as an active substance in a pharmaceutical product called Ibuprofen Grindex®, reacted completely with an α-naphthylamine 0.04 % solution, in the presence of sodium nitrite $NaNO_2$ 4% aqueous solution, by heating 5 minutes at 50-60°C, followed by a forced cooling for 10 minutes on an ice bath, that led to the quantitative production of a bright orange colored compound with a yellowish shade (an azo-dye), which was obtained in an amount perfectly equivalent to Ibuprofen present in the studied sample. By spectrophotometric analysis of the obtained azo-dye at the wavelength corresponding to its absorption maximum $\lambda_{max.}$ = 458 nm, Ibuprofen in the analyzed, sample was directly spectrophotometrically measured, in relation to the absolute ethanol p.a. as a control (Fig. 2).

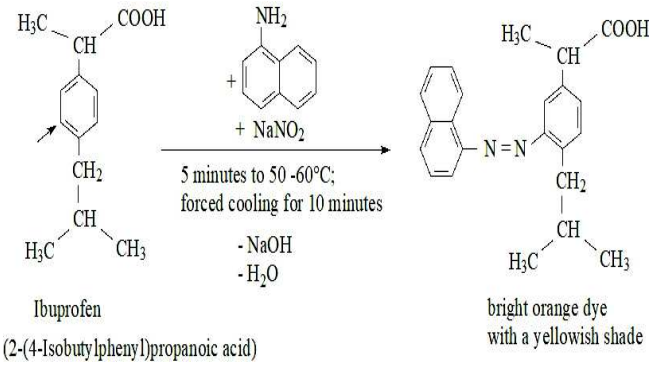

Fig. 2. Chemical reaction pathway of Ibuprofen assigned to the synthesis of a bright orange dye with a yellowish shade

The absorbances of ten standard solutions (0.75 µg-mL – 15.00 µg/mL) were measured. These solutions have been prepared directly from the standard working solution 150 µg/mL according to Table I. Final solutions volume in each graduated test tube was 20 mL, after making up to the mark with absolute ethanol (TABLE I).

TABLE I. Obtaining the set of standard ibuprofen solutions from standard working solution

mL Ibuprofen standard working solution, 150 µg/mL	**Required reagents added to standard solutions**		
	mL alpha-naphthylamine, 0.04 %	***mL sodium nitrite, $NaNO_2$, 4 %***	***mL absolute ethanol***
0.1	0.5	0.3	19.1
0.2	0.5	0.3	19.0
0.3	0.5	0.3	18.9
0.4	0.5	0.3	18.8
0.6	0.5	0.3	18.6
0.8	0.5	0.3	18.4
1.0	0.5	0.3	18.2
1.2	0.5	0.3	18.0
1.6	0.5	0.3	17.6
2.0	0.5	0.3	17.2

B.1. Ibuprofen sample preparation and calculation procedure.

Average weight of a pharmaceutical tablet was mc = 0.5661 g = **566.1 mg**. The official active substance content of pure ibuprofen on the pharmaceutical tablet was **400 mg.** a = 0.0516 g of ibuprofen powder and passed with 10 mL of absolute ethyl alcohol in a Berzelius beaker. The obtained solution was transferred into a volumetric flask of volume V = 100 mL. A volume of v = 0.3 mL was measured, into a graduated test tube of volume V_P = 20 mL and α-naphthylamine solution 0.04% a $NaNO_2$, 4% were added (table II). It was heated on a water bath to 50-60 ºC at constant temperature for 5 minutes. The sample solution was kept on an ice bath for 10 minutes and then filled with absolute ethanol to the mark. (TABLE II). Mean sample absorbance was Ap = 0.273.

TABLE II. PREPARATION OF THE SAMPLE IBUPROFEN SOLUTION

Sample solution of Ibuprofen Grindex® **(mL)**	**Reagents added to the sample solution**		
	mL alpha-naphthylamine, 0.04 % solution	***mL sodium nitrite, $NaNO_2$, 4 % aqueous***	***mL absolute ethanol***

		solution	
0.3	0.5	0.3	18.9

B.2. Statistic analysis.. The aim was to determine the following parameters: the linearity of the proposed method, detection limit (LD), quantitation limit (LQ), stability of the standard solutions, and system precision that consisted of the standard solutions containing pure Ibuprofen and the UV-VIS spectrophotometer utilized for measurements, as a whole.

Linearity of the proposed method. The linearity of an analysis process consisted of the ability to lead to results directly proportional to the concentration of an analyte in a given sample, within a given range (0.75 µg/mL-15.00 µg/mL). The correlation coefficient had to be R > 0.999 and linear regression coefficient $R^2 \geq 0.999$. Microsoft Office Excel 2016 software was used (TABLE III).

TABLE III. STATISTICAL PARAMETERS OF THE LINEARITY

Observations (measured absorbance values)	**Regression Statistics**			
	Correlation coefficient (Multiple R)	***Linear regression coefficient R square, R^2)***	***Adjusted R square (adjusted R^2)***	***Standard error of the linear regression (SE)***
10	0.999676	0.999352	0.999271	0.004974

Detection limit (LD) and quantitation limit (LQ), *Detection limit (LD*) was the smallest amount of analyte that could be detected in a sample compared to a blank. It was evaluated using the formula: LD = 3. SE/ slope (A). SE has represented standard error of the regression line. The *Quantitation limit (LQ)* was given by the lowest analyte concentration in a sample, which could be quantified. Its value was calculated as follows: LQ = 10. SE / slope (B).

Stability of the prepared standard solutions A standard solution with a concentration of 5.0 µg/mL was chosen from the set of ten standard solutions prepared (from Table I). This solution was investigated for 32 hours at λ = 458 nm from the time of its preparation, at various intervals, in normal storage conditions. Relative standard deviation (coefficient of variation) RSD %: RSD % = (SD. 100)/ $X_{Average}$ (C). For accurate measurements, RSD ≤ 5%. (TABLE IV).

TABLE IV. STABILITY ANALYSIS OF IBUPROFEN STANDARD SOLUTIONS

Number of experimental trials	**Stability over time of the standard solutions containing pure Ibuprofen**				
	Time (measured in hours)	***Mean measured absorbance A(λ)***	**The mean value**	***Standard deviation (SD)***	***Relative standard deviation (RSD %)***
1.	0	0.271	0.2671	0.003871	1.4493
2.	2	0.270			
3.	4	0.270			
4.	6	0.268			
5.	12	0.269			
6.	18	0.266			
7.	24	0.263			
8.	32	0.260			

System Precision. For system precision investigation, a target standard solution of 1.50 µg./ mL was chosen from the standard solutions set (from Table I) and processed under established experimental conditions. Standard deviation (SD) and RSD% were calculated. RSD ≤ 5% (TABLE V).

TABLE V. SYSTEM PRECISION ANALYSIS

Number of experimental trials	**System precision, as a whole, that consists of the standard solutions containing pure Ibuprofen and the UV-VIS spectrophotometer**				
	Standard solution of Ibuprofen, 1.50 µg/mL	***Mean measured absorbance A(λ)***	**The mean value**	***Standard deviation (SD)***	***Relative standard deviation (RSD %)***
1.		0.271	0.1146	0.003050	2.6614
2.		0.270			
3.		0.270			
4.		0.268			
5.		0.269			

III. RESULTS

a. Calculation of Ibuprofen concentration Cp (µg/mL) taken into study, from the calibration graph (Fig. 3).

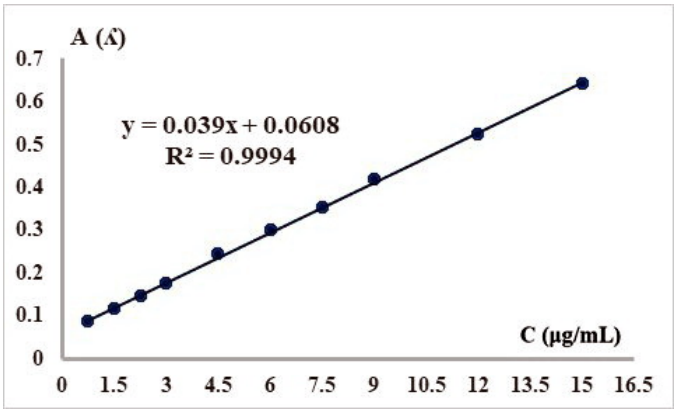

Fig. 3 Calibration graph obtained for Ibuprofen standard solutions (0.75 µg/mL -15.00 µg/mL)

From the regression line y = 0.039 x + 0.0608 (Fig, 3), y = Ap = 0.273 and x = Cp (µg/mL). Thus, Cp (µg/mL), = (0.273 – 0.0608)/0.039 (1). Cp = 5.441 µg/mL pure Ibuprofen. Ap = 0.273 = mean absorbance of the sample solution.

b. Calculation of Ibuprofen pure content (X µg) from V_P = 20 mL solution existing in the graduated test tube, depending on C_P (µg/mL) found above: C_P µg →1 mL solution, so in V_P = 20 mL solution were X = 20. C_P µg of Pure Ibuprofen (2). X = 20. 5.441. So, X = 108.82 µg of Ibuprofen.

c. Ibuprofen pure content estimation (Y µg) from the initial solution existing in V = 100 mL volumetric flask, depending on X (µg) value: If X µg Ibuprofen → v = 0.3 mL sample solution, then in V = 100 mL volumetric flask were Y = (V. X) / v µg pure Ibuprofen (3). Y = (100. 108.82) / 0.3. Thus, Y = 36273.33 µg pure Ibuprofen

d. Ibuprofen pure content evaluation (Y1 µg transformed in mg), calculated on a pharmaceutical tablet, as a final result, reported to mc = 0.5661 g = 566.1 mg: It is known that: Y (µg) pure Ibuprofen → a = 0.0516 g ibuprofen powder, then in mc = 0.5661 g average weight of a pharmaceutical tablet were: Y1 = (mc. Y) / a Ibuprofen (4). Y1 = (0.5661 36273.33) / 0.0516. Thus, **Y1 = 397952.173 µg** of pure Ibuprofen / pharmaceutical tablet = **397.952 mg of pure Ibuprofen/ pharmaceutical tablet, as a final result** (TABLE VI).

TABLE VI. CALCULTATION OF IBUPROFEN PURE CONTENT BY FILM-COATED TABLET

Analyzed sample solution of Ibuprofen	Calculated parameters			
	Mean measured absorbance (A_P)	***Sample solution concentration (µg/mL)***	***(µg) Ibuprofen content / film-coated tablets***	***(mg) Ibuprofen content / film-coated tablets***
Ibuprofen Grindex® tablets	0.273	5.441	397952.173	397.952

e. Percentage content (Z%) estimation of pure ibuprofen in film-coated tablet, depending on Y1 from above expressed in mg and reported to the pure official content of ibuprofen on the pharmaceutical tablet, that was 400 mg:

For 100 % content → 400 mg of pure Ibuprofen, then for Y1 (mg) Ibuprofen it was: Z = (Y1 .100) / 400 % = (Y1 / 4) % (5). It was concluded that: Z = 397.952 / 4 = 99.488 %. Thus, **Z = 99.488 %** of Pure Ibuprofen percentage content / pharmaceutical tablet.

Statistical analysis results. ***Linearity of the method.*** Spectrophotometric analysis of Ibuprofen has shown very good linearity, regression coefficient value was $R^2 = 0.9994$ (fig. 4). $R^2 \geq 0.9990$. The standard error of the regression line (SE) was SE = 0.004974, which had a corresponding, highly low value (Table III).

Detection limit (LD) and quantitation limit (LQ) were calculated with formulas (A) and (B) written above. LD = **0,383 µg/ mL** and LQ = **1,275 µg/ mL**, have been within the normal limits. ***Stability of the standard solutions.*** Following the calculation, it was found that the value of the SD standard deviation = 0.003871, and the relative standard deviation was calculated with the formula (C) as follows: RSD = (0.003871. 100) / 0.2671 = 1.4493%. Thus, RSD = 1.4493%. It was found, according to table IV, that the solutions were stable during at least 32 hours from their preparation.

System Precision estimation The absorbances corresponding to the chosen standard solution 1.50 µg/ mL proved to be very close to each other (Table VI). According to formula (C) the relative standard deviation RSD = (0.003050. 100) / 0.1146 = 2.6614%. Thus, RSD = 2.6614%, was within the normal limits, RSD ≤. 5% (Table V).

IV. CONCLUSIONS

It was found an amount of 397.952 mg of pure Ibuprofen content / film-coated tablet. This value was very close to Ibuprofen content declared by the pharmaceutical manufacturer (400 milligrams), with a mean percentage deviation of only 0.512 % from the officially declared amount of active substance and felt within the normal limits (< 5%).

The applied analysis method was linear over the entire chosen concentration range of 0.75 µg / mL -15.00 µg / mL. $R^2 = 0.999352$ $R^2 \geq 0.9990$ and the correlation coefficient R = 0.999676, R > 0.9990 have been fit perfectly in the normal range of values. LD = 0,383 µg/ mL and LQ = 1,275 µg/ mL. Aqueous solution of 5.0 µg/mL Ibuprofen was particularly stable and could be stored at least 32 hours, the value of the relative standard deviation RSD = 1.4493%, RSD ≤ 5%. The system composed by the standard solutions of Ibuprofen and UV-Vis Spectrophotometer presented statistically a very good precision assigned by the standard relative deviation value, which was RSD = 2.6614%, RSD ≤ 5%. The proposed method can be successfully applied in practice to quantitative Ibuprofen analysis from different pharmaceutical tablet forms, according to the actual European and international standards.

ACKNOWLEDGMENT

This study is simply an objective scientific research paper that does not aim to confirm or deny the official results obtained by the pharmaceutics manufacturing company, nor to cause any damage to its image. We, the authors, declare no conflict of interests.

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