Investigation of linearity, detection limit (LD) and quantitation limit(LQ) of active substance from pharmaceutical tablets

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The aim of this research was to exactly quantify pure sodium metamizole from tablets , using a spectrophotometric analysis in Visible range. The method applied has been subjected to a validation protocal which consisted in analyzing the following parameters: linearity of the method, detection limit (LD) , quantitation limit (LQ).

Following actual dosing, pure sodium metamizole amount in tablet of pharmaceutical was found to be 477.477 mg assigned to a percentage content of 95.495 %, very close to official declared amount (500 mg), with an maximum average percentage deviation of only 4.505 % from the official declared active substance content. This value was situated below the maximum admissible percentage deviation from stated active substance content (± 5%), established by Romanian Pharmacopoeia, X-th Edition rules.

Keywords: sodium metamizole, detection limit, quantitation limit

Metamizole (dipyrone, as sodium salt) is a popular analgesic medicine, non-opioid drug, commonly used in human and veterinary medicine similar to other natural or synthetic active principles such as black pepper, lavender, pepper, curcumin ibuprofen [1-6]. In some cases, this agent is still incorrectly classified as a non-steroidal anti-inflammatory drug (NSAID). Apart from its strong *analgesic effect*, the medication is an moderate *antipyretic* and significant *spasmolytic agent* [7-12]

The spasmolytic effect of metamizole is a result of mechanism associated with a powerfull inhibition of intracellular calcium (Ca^{2+}) release, as a result of the reduced inositol phosphate synthesis. Metamizole is predominantly applied in the therapy of pain of different etiology, of spastic conditions, especially affecting the digestive tract[13], and of fever refractory to other treatments. It is especially indicated as an strong, effective analgesic in all types of moderate and intense pain (neuralgia, arthralgia, myalgia, headache, dysmenorrhoea), including postoperative pain, renal and biliary colic, dental pain[14-17]

Experimental part

Method and procedures

Algocalmin(sodium metamizole) was oxidized by 5.0 % ammonium *ortho*molybdate $(NH_4)_2MoO_4$ aqueous solution in a strongly acidic medium (H_2SO_4, 40%) , to form a bluish-colored green compound that showed a maximum absorbtion to λ = 690 nm (fig 1).

Visible absorption spectra of green-bluish compound synthetized. Evaluation of maximum absorption wavelength, specific absorptivity and molar absorption coefficient

Sample synthesis from Algocalmin® Zentiva tablets

One Algocalmin tablet was weighted to find the average mass of pharmaceutical product It was found that mean mass value was m_C = 0.5333 g. The official declared content by pharmaceutical company of pure sodium metamizole in tablet was 500 mg. Then, 3 tablets were finely crushed and a = 0.1102 g of Algocalmin obtained powder were quantitatively brought with a little volume of absolute methanol (8 mL) into a V_1 = 100 mL volumetric flask. The content was mixed until complete dissolution of sodium metamizole and filled up to the mark with distilled water.

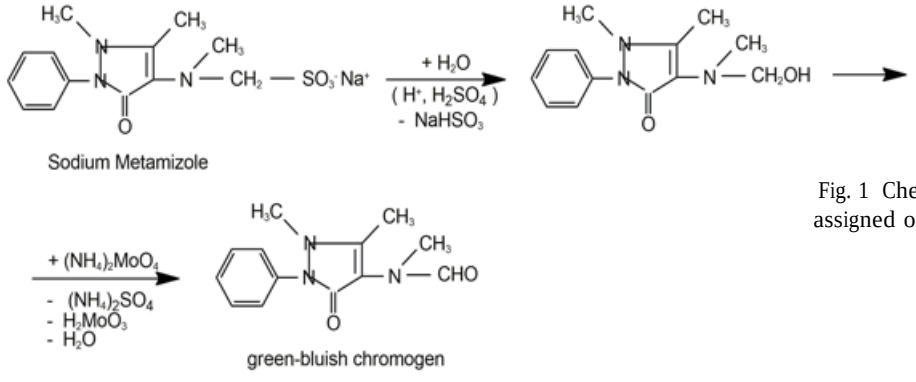

Fig. 1 Chemical reactions of Sodium Metamizole assigned of green-bluish chromogen synthesis

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From the obtained sample solution, v_1 = 0.4 mL were measured and quantitatively brought to 10 mL graduated glass tube. Then, 1.5 mL of ammonium orthomolybdate $(NH_4)_2MoO_4$, 5.0 % and 0.5 mL H_2SO_4, 40% were added. (table 3). Sample solution was stirred well, stored in a dark place for 30 minutes and filled up to volume V_P = 10 mL with distilled water.

Five measurements have been made and sample mean absorbance Ap, was calculated. (T. 3).

Calculation method

Calculation of pure sodium metamizole amount in pharmaceutical tablet

The average measured mass of one tablet of pharmaceutical product was m_C = 0.5333 g (533,3 mg). According to manufacturing company, a pharmaceutical tablet of Algocalmin® contained 500 mg of pure sodium metamizole. The amount of pure sodium metamizole existing in final volume of sample solution (V_P) was determined as follows: $X = C_S$ (µg/mL) .V_P (2) , whereas V_P = 10 mL has represented sample solution final volume contained in graduated glass tube.

The quantity of pure sodium metamizole from V_1 = 100 mL (volumetric flask) was calculated : $X_1 = (V_1 . X) / v_1$ (3), v_1 = 0.4 mL was sample solution volume measured from volumetric flask and quantitatively brought to V_P = 10 mL graduated test tube.

The amount of pure sodium metamizole in tablet of Algocalmin® was investigated as follows: $Y_1 = (m_C . X_1) / a$ (4) , whereas a = 0.1102 g fine powder sample of Algocalmin®. prepared from pharmaceutical tablets.Y_1 has represented the amount of pure sodium metamizole in tablet sample, expressed as µg pure sodium metamizole / tablet, then by transforming into mg pure sodium metamizole / tablet of Algocalmin®[18-25]

Table 1

MAXIMUM PERCENTAGE DEVIATIONS FTOM THE STATED CONTENT OF ACTIVE SUBSTANCE IN PHARMACEUTICALS

Declared content of active substance	Maximum accepted percentage deviations
up to 10 mg	± 10 %
10 mg and up to 100 mg	± 7,5 %
100 mg and over 100 mg	± 5 %

*Percentage content of pure sodium metamizole in commercial tablet (Z %):*if it was known that one tablet of Algocalmin® had 500 mg of pure sodium metamizole*:*

$Z = (100 . Y_1) / 500$ (%) , so $Z = Y_1 / 5$ (%) (5), Y_1 has been expressed in mg .

Linearity of the method. Regression line parameters. The linearity of 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 (1-40µg/mL), Practically, the intensity of analytical signal (measured absorbance) has varied in directly proportion to the concentration, for a given area. Correlation coefficient had to be R > 0.999 and linear regression coefficient R^2 e™ 0.999 [10, 11]. The statistic parameters of method linearity were then determined, using Microsoft Office Excel 2016 software and described in table 4.

Detection limit (LD) was the smallest amount of analyte that could be detected in a sample compared to a blank, under established experimental conditions. It was expressed in the same units as concentration of the analyte (ìg/mL) and was evaluated using formula: LD = 3. SE/ slope (6). SE has represented standard error of the regression line [18-26]

Quantitation limit (LQ) was given by the lowest analyte concentration in a sample, which could be quantified (determined) with acceptable precision and accuracy under the same experimental conditions. Its value was expressed in the same units as analyte concentration (ìg/mL) and was calculated as follows: LQ = 10. SE / slope (7) [18-27].

Results and discussions

Visible absorption spectra of green-bluish compound. Maximum absorption wavelength, specific absorptivity and molar absorption coefficient investigation

Absorption spectra of green-bluish chromogen was plotted for 7 µg/mL solution (fig. 2) and maximum absorption wavelength was determined to λ = 690 nm. Mean measured absorbance value to this wavelength was A = 0.1653. Concentration of 7 µg/mL was transformed as follows: 7 µg/mL = 0.0007 g/100 mL and was assigned to a $2.853.10^{-5}$ mole/L concentration, with the respect of calculated molecular weight corresponded to green-bluish chromogen, which was M = 245.338 g/mole (fig.3).

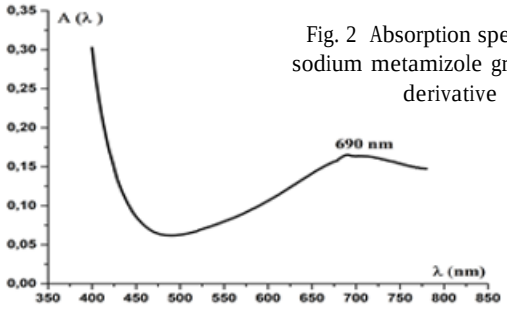

Fig. 2 Absorption spectra (VIS) of sodium metamizole green-bluish derivative

Specific absorptivity:

According to Lambert-Beer's law: $A^{1\%}_{1cm} = A / C$ (g / 100 mL) (1), C = 0.0007 g /100 mL = solution concentration

A = calculated mean absorbance = 0.1653

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

By replacing these values in relation (1), it was obtained: $A^{1\%}_{1cm} = 0.1653 / 0.0007 = 236.143$. Thus, $A^{1\%}_{1cm} = 236.143$

Molar absorption coefficient: Similarly, $E_{molar} = A / C$ (mole /L) (2), $C = 2.853 .10^{-5}$ mole / L = solution concentration

$M_{green\text{-}bluish\ compund} = 245.338$ g/mole

A = calculated mean absorbance = 0.1653

E_{molar} = molar absorption coefficient

By replacing these values in relation (2), it was obtained: $E_{molar} = 0.1653. 10^5 / 2.853 = 5793.901$. Thus, $E_{molar} = 5793.901$

Fig. 3 Specific absorptivity and molar absorption coefficient calculation

Table 2

MEASURED ABSORBANCES OF SODIUM METAMIZOLE STANDARD SOLUTUONS

No. det	mL sodium metamizole 100 µg/mL (standard)	mL $(NH_4)_2MoO_4$ 5%	mL H_2SO_4, 40%	C (µg/mL)	A(Å)
1.	0.1	1.5	0.5	1.0	0.0130
2.	0.2	1.5	0.5	2.0	0.0432
3.	0.3	1.5	0.5	3.0	0.0708
4.	0.4	1.5	0.5	4,0	0,0920
5.	0.5	1.5	0.5	5.0	0.1180
6.	1.0	1.5	0.5	10.0	0.2360
7.	1.5	1.5	0.5	15.0	0.3440
8.	2.0	1.5	0.5	20.0	0.4620
9.	2.5	1,5	0.5	25.0	0.5800
10.	3.0	1.5	0.5	30.0	0.7080
11.	3.5	1.5	0.5	35.0	0.8120
12.	4.0	1.5	0.5	40.0	0.9350

Table 3

SAMPLE CONCENTRATION AND SODIUM METAMIZOLE PURE AMOUNTS DETERMINED IN TABLET

Algocalmin® sample	A_S	C_S (µg/mL)	µg sodium metamizole /tablet	mg sodium metamizole /tablet
	0.9204	39.466	477477.717	**477.477**

Linearity of the method -regression line drawing and characteristics

Determined absorbances values of standard solutions measured to λ= 690 nm, were listed in table 2.

Investigation of sodium metamizole concentration (µg/mL) in Algocalmin® sample solution

Mean absorbance of Algocalmin® sample containing sodium metamizole as active substance, sodium metamizole concentration which was expressed in µg/mL, as well as the amount of pure sodium metamizole calculated in tablet of pharmaceutical product, were shown in table 3.

From relation (1): $C_S = (A_S + 0.0031) / 0.0234$ (µg/mL) = (0.9204 + 0.0031)/ 0.0234, so C_S = 39.466 µg/mL has represented sample concentration of pure sodium metamizole.

Calculation of pure sodium metamizole amount on pharmaceutical tablet Algocalmin®

Pure sodium metamizole amount existing in final volume of sample solution (V_p = 10 mL) according to equation (2), was: X = 39.466 . 10 = 394,66 µg.

Sodium metamizole quantity from V_1 = 100 mL (volumetric flask) was calculated , as follows: X_1 = (100. 394.66) /0.4, according to relation (3). So, X_1 = 98665 µg.

The amount of pure sodium metamizole in tablet of pharmaceutical product : Y_1 = (0.5333 .98665)/ 0.1102 = 477477.717µg sodium metamizole. Thus, Y_1 = 477,477 mg pure sodium metamizole / tablet of pharmaceutical Algocalmin® .

Percentage content of pure sodium metamizole in commercial tablet (Z %): it is known that one tablet of Algocalmin® had a content of 500 mg pure sodium metamizole, so Z = 477,477 / 5 = 95.495 % revealed by equation (5). Thus, Z = 95.495% has expressed sodium metamizole percentage contents in tablet.

Calculated pure sodium metamizole value has represented 95.495% from the officially declared value (500 mg) by the pharmaceutical company and it had an average maximum percentage deviation of only 4.505% from the official pure declared active substance content.

Regression statistic parameters evaluation

Statistic parameters of method linearity which have been determined in Microsoft Excel 2016, were presented in table 4.

Table 4

STATISTIC VALUES OF LINEAR REGRESSION PARAMETERS

Regression Statistics	
Multiple R (Correlation coefficient)	0.999898
R Square R^2(Linear regression coefficient)	0.999795
Adjusted R Square R^2	0.999775
Standard Error (SE)	0.004882
Observations	12

Ecuation of the regression line was: y = 0.0234.x - 0.0031, or A_S (λ) = 0.0234. C_S (µg/mL) - 0.0031. The intercept was (-) 0.0031 and slope : 0.0234. Linear regression coefficient was $R^2 \geq 0.999$ and correlation coefficient R > 0,999 were above minimum admisible value (table 3) and were situated within the normal range of values, which demonstrated the linear variation of measured standard solutions absorbances coresponding to their concentrations. Standard error of the regression line (SE) was SE = 0.004882 (table 3) had a coresponding, highly low value.

Detection limit (LD) and quantitation limit (LQ); Detection limit was, LD = 3 .0.004882 / 0.0234 , thus LD = 0.626 µg/mL. according to relation (6) and Quantitation limit was assigned with LQ = 10 .0.004882 / 0.0234, so LQ = 2.086 µg/mL, from equation (7).

Conclusions

The method used for Visible spectrophotometric analysis of sodium metamizole in tablets marketed under the name *Algocalmin Zentiva*®, was linear in standard concentrations range 1 Eg/mL -40 Eg/mL; the linear regression coefficient was R^2 = 0.999795, $R^2 \geq 0.999$ and corelation coefficient R = 0.999898, R > 0.999 . Standard error of the regression line SE = 0.004882, detection limit LD = 0.626 Eg/mL and quantitation limit LQ = 2.086 Eg/mL were located within the normal range of values.

Visible spectrophotometric (VIS) method used for quantitative analysis of sodium metamizole in tablets has been successfully validated through the complete scrolling of studied stages and could be applied in practice to active substance dosing from different samples.

New methods for detecting the active principles in the various of pharmaceutical forms with spectacular results are *Ordered mesoporous carbon based sensor for Sensitive detection* or Electrochemical determination [28-29].

Acknowledgments . This study is simply a scientific research paper that does not aim to confirm or deny the official results of pharmaceutical manufacturing company, nor to cause any image damage.

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