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 protocol 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 powerful inhibition of intracellular calcium (Ca2+) 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 a 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 (NH₄)₂MoO₄ aqueous solution in a strongly acidic medium (H₂SO₄, 40%) , to form a bluish-colored green compound that showed a maximum absorption 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 mₑ = 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 powder were quantitatively brought with a little volume of absolute methanol (8 mL) into a V₁ = 100 mL volumetric flask. The content was mixed until complete dissolution of sodium metamizole and filled up to the mark with distilled water.

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From the obtained sample solution, \( v_1 = 0.4 \text{ mL} \) were measured and quantitatively brought to 10 mL graduated glass tube. Then, 1.5 mL of ammonium orthomolybdate \((\text{NH}_4)_2\text{MoO}_4, 5.0 \%\) and 0.5 mL \( \text{H}_2\text{SO}_4, 40\% \) were added. (table 3). Sample solution was stirred well, stored in a dark place for 30 minutes and filled up to volume \( V_p = 10 \text{ mL} \) with distilled water.

Five measurements have been made and sample mean absorbance \( A_p \), 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 \( m_C = 0.5333 \text{ g} \) (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: \( X = C_s (\mu g/\text{mL}) \cdot V_p \), whereas \( V_p = 10 \text{ mL} \) has represented sample solution final volume contained in graduated glass tube.

The quantity of pure sodium metamizole from \( V_1 = 100 \text{ mL} \) (volumetric flask) was calculated : \( Y_1 = (m_C \cdot X) / a \), whereas \( a = 0.1102 \text{ g} \) fine powder sample of Algocalmin®, prepared from pharmaceutical tablets, \( Y_1 \) has represented the amount of pure sodium metamizole in tablet sample, expressed as \( \mu g \) pure sodium metamizole / tablet of Algocalmin®

**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 \( \mu g/\text{mL} \) solution (fig. 2 ) and maximum absorption wavelength was determined to \( \lambda = 690 \text{ nm} \). Mean measured absorbance value to this wavelength was \( A = 0.1653 \). Concentration of 7 \( \mu g/\text{mL} \) was transformed as follows: 7 % \( \mu g/\text{mL} = 0.0007 \text{ g}/100 \text{ mL} \) and was assigned to a 2.853 \( \times 10^{-5} \) mole/L concentration, with the respect of calculated molecular weight corresponded to green-bluish chromogen, which was \( M = 245.338 \text{ g/mole} \) (fig.3).

**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 (1-40\( \mu g/\text{mL} \) ). 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 \( R^2 \) = 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 \( (\mu g/\text{mL}) \) and was evaluated using formula: \( LD = 3 \cdot SE / \text{slope} \) (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 \( (\mu g/\text{mL}) \) and was calculated as follows: \( LQ = 10 \cdot SE / \text{slope} \) (7) [18-27].
Linearity of the method - regression line drawing and characteristics

Determined absorbances values of standard solutions measured to $\lambda = 690$ nm, were listed in Table 2.

Investigation of sodium metamizole concentration ($\mu g/\text{mL}$) in Algocalmin® sample solution

Mean absorbance of Algocalmin® sample containing sodium metamizole as active substance, sodium metamizole concentration which was expressed in $\mu g/\text{mL}$, as well as the amount of pure sodium metamizole calculated in tablet of pharmaceutical product, were shown in Table 3.

From relation (1): $C_S = (A_S + 0.0031) / 0.0234 \text{ (} \mu g/\text{mL}) = (0.9204 + 0.0031)/0.0234$, so $C_S = 39.466 \mu g/mL$ 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 ($V_p = 10 \text{ mL}$) was calculated, as follows: $X_p = (100 \times 39.466)/0.4 = 3946.6 \mu g/mL$.

The amount of pure sodium metamizole in tablet of pharmaceutical product: $Y = (0.5333 \times 39.465)/0.1102 = 199.47717 \mu g$ sodium metamizole. Thus, $Y = 199.477 mg$ 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 $\mu g$ pure sodium metamizole, so $Z = 199.477 / 500 = 0.39895$ % revealed by equation (5). Thus, $Z = 39.895%$ has expressed sodium metamizole percentage contents in tablet.

Calculated pure sodium metamizole value has represented 95.495% from the officially declared value ($500 \mu g$) 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 2

| No. | C (µg/mL) | A (º) |
|-----|-----------|-------|
| 1. | 0.1       | 1.5   |
| 2. | 0.2       | 1.5   |
| 3. | 0.3       | 1.5   |
| 4. | 0.4       | 1.5   |
| 5. | 0.5       | 1.5   |
| 6. | 1.0       | 1.5   |
| 7. | 1.5       | 1.5   |
| 8. | 2.0       | 1.5   |
| 9. | 2.5       | 1.5   |
| 10.| 3.0       | 1.5   |
| 11.| 3.5       | 1.5   |
| 12.| 4.0       | 1.5   |

Table 3

| Algocalmin® sample | Ax | Cx (µg/mL) | mg sodium metamizole/tablet |
|--------------------|----|------------|-----------------------------|
| 0.9204             | 39.465 | 477477.717 | 477.477                     |

Table 4

| Regression Statistics | Multiple R (Correlation coefficient) | 0.999938 |
|-----------------------|-------------------------------------|----------|
| R Square R² (Linear regression coefficient) | 0.999975 |
| Adjusted R Square R²* | 0.999975 |
| Standard Error (SE) | 0.004882 |
| Observations | 12 |

Euation of the regression line was: $y = 0.0234 \times x - 0.0031$, or $A_S (\lambda) = 0.0234 \times C_S (\mu g/mL) - 0.0031$. 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 admissible value (Table 3) and were situated within the normal range of values, which demonstrated the linear variation of measured standard solutions absorbances corresponding to their concentrations. Standard error of the regression line (SE) was $SE = 0.004882$ (Table 3) had a corresponding, highly low value.

Detection limit (LD) and quantitation limit (LQ): Detection limit was $LD = 3 \times 0.004882 / 0.0234$, thus $LD = 6.26 \mu g/mL$ according to relation (6) and Quantitation limit was assigned with $LQ = 10 \times 0.004882 / 0.0234$, so $LQ = 2.086 \mu g/mL$, 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 $1 \text{ Eg/mL - 40 Eg/mL}$; the linear regression coefficient was $R^2 = 0.999795$, $R^2 \geq 0.999$ and correlation coefficient $R = 0.999989$, $R > 0.999$. 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 scrilling 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].

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