

THE SPECTROPHOTOMETRIC ANALYSIS METHOD OF METAMIZOLE FROM PHARMACEUTICAL TABLETS: INVESTIGATION OF LINEARITY, LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

METODA DE ANALIZĂ SPECTROFOTOMETRICĂ A METAMIZOLULUI DIN TABLETELE FARMACEUTICE: INVESTIGAREA LINEARITĂȚII, A LIMITEI DE DETECȚIE ȘI A LIMITEI DE CUANTIFICARE

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Abstract. The aim of this research was to exactly quantify the pure sodium metamizole content from pharmaceutical tablets, by using a spectro-photometric analysis method in visible range. The method applied has been subjected to a validation protocol which consisted in analyzing the following parameters: linearity of the method, limit of detection (LD) and limit of quantification (LQ). The pure sodium metamizole amount in a pharmaceutical tablet was found to be 477.477 mg, assigned to a percentage content of 95.495 %, close to official declared amount (500 mg), presenting an average percentage deviation of 4.505 % from the officially stated active substance content. This value was situated below the maximum admissible percentage deviation of active substance content ($\pm 5\%$), stated by Romanian Pharmacopeia, Xth Edition.

Key words: sodium metamizole, linearity, detection limit, quantitation limit

Rezumat. Lucrarea și-a propus să cuantifice conținutul de metamizol de sodiu pur din tabletele farmaceutice, utilizând o metodă de analiză spectrofotometrică în domeniul vizibil. Metoda aplicată a fost supusă unui protocol de validare care a constat în determinarea următorilor parametri: linearitatea metodei, limita de detecție (LD) și limita de cuantificare (LQ). Cantitatea de metamizol de sodiu pur determinată într-o tabletă farmaceutică a fost de 477,477 mg, corespunzător unui conținut procentual de 95,495%, cu o deviație medie de 4,505% față de conținutul de substanță activă declarat oficial (500 mg), valoare sub deviația procentuală maxim admisibilă a conținutului de substanță activă ($\pm 5\%$) stabilit prin normele din Farmacopeea Română, Ediția a X^{-a}.

Cuvinte cheie: sodiu metamizole, linearitate, limită de detecție, limită de cuantificare

INTRODUCTION

Metamizole (sodium salt of dipyron) is a popular analgesic medicine, non-opioid drug, commonly used in human and veterinary medicine. Apart from its

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strong analgesic effect, the medication is a moderate antipyretic and significant spasmolytic agent (Farmacopeia X 1993; Nita *et al.*, 2018). The spasmolytic effect of metamizole is a result of mechanism associated with a powerful 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, as well as of the refractory fever to other treatments. It is especially indicated as a strong, effective analgesic in all types of moderate and intense pain (neuralgia, arthralgia, myalgia, headache, dysmenorrhea), including postoperative pain, renal and biliary colic, dental pain (Jasiecka *et al.*, 2018; Brune *et al.*, 2010; Chandrasekharan *et al.*, 2002).

MATERIAL AND METHOD

Sodium metamizole was oxidized by 5.0 % ammonium ortho-molybdate $(\text{NH}_4)_2\text{MoO}_4$ aqueous solution in a strongly acidic medium (H_2SO_4 , 40%), to form a bluish-colored green compound (fig. 1), with a maximum absorption at $\lambda = 690 \text{ nm}$ (Dorneanu *et al.*, 2003, Dorneanu *et al.*, 2007).

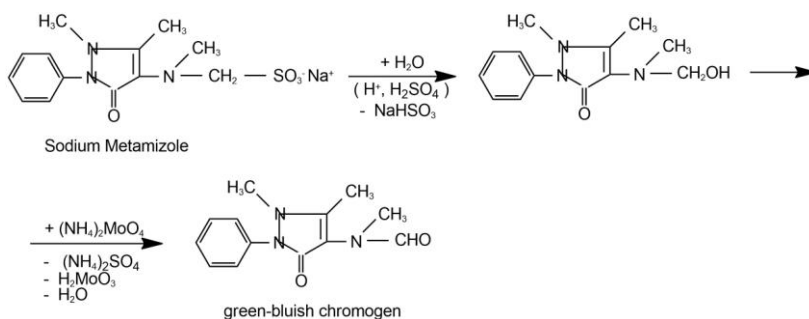


Fig. 1 Chemical reactions of sodium metamizole assigned of green-bluish chromogen

Three pharmaceutical tablets containing metamizole as active substance, with the average mass of 0.5333 g/tablet and official declared 500 mg pure sodium metamizole/tablet, were crushed and the obtained powder (0.1102 g) was quantitatively brought with a little volume of absolute methanol (8 mL) into a $V_1 = 100 \text{ mL}$ volumetric flask. The content was mixed until complete dissolution of sodium metamizole and filled up to the mark with distilled water. 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 ortho-molybdate $(\text{NH}_4)_2\text{MoO}_4$, 5.0 % and 0.5 mL H_2SO_4 , 40% were added. 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. The mean absorbance (Ap) of five measurements was calculated.

Linearity is the method's ability to obtain test results, which are directly proportional to the concentration of analyte in the sample (Roman *et al.*, 1998). Practically, the intensity of the measured absorbance was directly proportional with the concentration between (1-40) $\mu\text{g/mL}$. The statistic parameters (Boiculescu *et al.*, 2007) characterizing the method linearity were determined, by using Microsoft Office Excel 2016 software.

Limit of detection (LD) is the smallest amount of analyte that could be detected in a sample as compared to a blank (Banjare *et al.*, 2013; Bhalani *et al.*, 2015; Mubeen *et al.*, 2009) under the same experimental conditions. Limit of quantification (LQ) is given by the lowest analyte concentration in a sample, that could be quantified (determined) with acceptable precision and accuracy (Muñoz *et al.*, 2015) under the same experimental conditions.

RESULTS AND DISCUSSIONS

Evaluation of sodium metamizole pure amount was based on the established regression plot line, presented in figure 2.

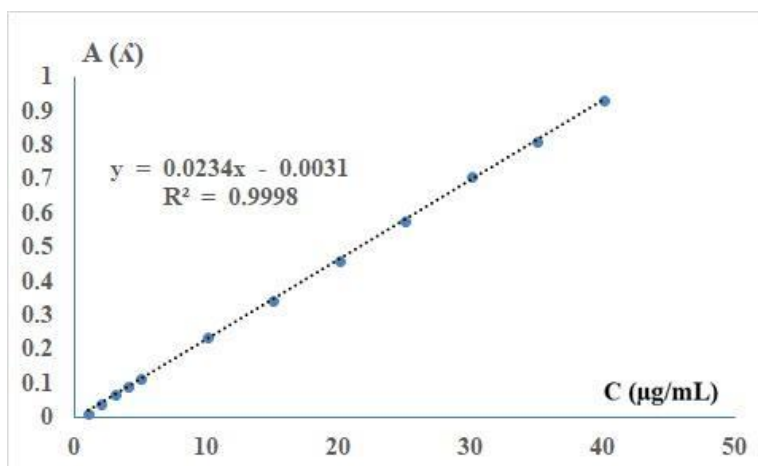


Fig. 2 Regression plot line of sodium metamizole standard solutions

By replacing y with the A_S (absorbance of the sample) and x with C_S (concentration of the sample) in the regression line equation $y = 0.0234x - 0.0031$, (Fig. 2), it will result: $A_S = 0.0234C_S - 0.0031$ and thus the concentration ($\mu\text{g/mL}$) of the sodium metamizole solution may be calculated according to equation 1:

$$C_S(\mu\text{g/mL}) = (A_S + 0.0031) / 0.0234 \quad (1),$$

whereas C_S is the concentration of the sample and A_S is the absorbance of the sample.

According to the manufacturing company, a pharmaceutical tablet should contain 500 mg of pure sodium metamizole. The amount of pure sodium metamizole existing in the final volume (V_P) was determined according to equation 2:

$$X = C_S/V_P \quad (2),$$

whereas $V_P = 10$ mL is the final volume of the sample solution.

The quantity of pure sodium metamizole from $V_1 = 100$ mL was determined according to equation 3:

$$X_1 = (V_1 \cdot X) / v_1 \quad (3),$$

whereas $v_1 = 0.4$ mL is the volume of sample solution measured from the volumetric flask and quantitatively brought to $V_p = 10$ mL graduated test tube.

The amount of pure sodium metamizole in a pharmaceutical tablet was determined according equation 4, as follows:

$$Y_1 = (m_c X_1) / a \quad (4),$$

whereas $a = 0.1102$ g is the quantity of powder sample prepared from the pharmaceutical tablets.

The content of pure sodium metamizole in the commercial tablet was calculated according to equation 5:

$$Z (\%) = Y_1 / 5 \quad (5),$$

whereas Y_1 is the amount of pure sodium metamizole, expressed as mg pure sodium metamizole/tablet.

The values of mean absorbance of the solution containing sodium metamizole, with concentration expressed in $\mu\text{g/mL}$, as well as the amount of pure sodium metamizole from a pharmaceutical tablet, are presented in table 1.

Table 1

Mean absorbance value, the corresponding concentration ($\mu\text{g/mL}$) of metamizole solution and the amount of metamizole from a pharmaceutical tablet

Mean absorbance value	C_s ($\mu\text{g/mL}$)	Amount of sodium metamizole(mg) /tablet
0.9204	39.466	477.477

Evaluation of sodium metamizole pure amount represented 95.495% from the value of 500 mg officially declared by the pharmaceutical company, thus the average percentage deviation of 4.505% from the officially declared content content, was within the allowance limit of variance (Table 2) according to the Romanian Pharmacopeia, Xth Edition.

Table 2

Maximum allowed percentage deviations from the stated content of active substance in pharmaceuticals stated by Romanian Pharmacopeia, Xth Edition

Declared content of active substance	Maximum accepted percentage deviations
up to 10 mg	$\pm 10 \%$
10 mg and up to 100 mg	$\pm 7.5 \%$
100 mg and over 100 mg	$\pm 5 \%$

The main statistic parameters used to evaluate the method linearity are presented in table 3. The resulted regression line equation was: $y = 0.0234.x - 0.0031$, or $A_S(\lambda) = 0.0234C_S (\mu\text{g/mL}) - 0.0031$. A strength correlation coefficient as well as a strength linear regression coefficient should be > 0.999 (Kapil Kalra, 2011; Aboud *et al.*, 2017). Linear regression coefficient being above minimum admissible value were within the normal range of values.

Table 3

Statistic values of linear regression parameters

Regression Statistics Coefficients	Value
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
Determinations	12

Limit of detection (LD) was expressed in the same units as the concentration of the analyte ($\mu\text{g/mL}$), being calculated according to equation (6):

$$LD = 3 SE / \text{slope} \quad (6),$$

whereas SE is the standard error of the regression line.

Limit of quantification (LQ) was calculated according to equation (7), being expressed in the same units as the concentration of the analyte ($\mu\text{g/mL}$):

$$LQ = 10 SE / \text{slope} \quad (7),$$

whereas SE is the standard error of the regression line.

The limit of detection (LD) was determined as being $LD = 0.626 \mu\text{g/mL}$, while the limit of quantification (LQ) was determined as being $LQ = 2.086 \mu\text{g/mL}$.

CONCLUSIONS

The visible spectrophotometric analysis method used for the evaluation of sodium metamizole in the pharmaceutical tablets was linear for the concentrations ranging between 1– 40 $\mu\text{g/mL}$, with a strength linear regression coefficient ($R^2 = 0.999795$) and correlation coefficient ($R = 0.999898$).

Standard error of the regression line ($SE = 0.004882$), limit of detection ($LD = 0.626 \mu\text{g/mL}$) and limit of quantification $LQ = 2.086 \mu\text{g/mL}$ were located within the normal range of values.

Visible spectrophotometric method used for quantitative analysis of sodium metamizole in pharmaceutical tablets was successfully validated to be applied in the practice of metamizole dosage from different samples.

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