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EVALUATION OF PRECISION AND ACCURACY OF A SPECTROPHOTOMETRIC METHOD FOR MEASURING CONCENTRATION OF ACTIVE COMPOUND IN PHARMACEUTICAL FORMS

EVALUAREA PRECIZIEI ŞI ACURATEȚII UNEI METODE SPECTROFOTOMETRICE DE MĂSURARE A CONCENTRAȚIEI COMPUSULUI ACTIV ÎN FORME FARMACEUTICE

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Abstract. *A spectrophotometric method in visible range measuring the active compound content of pharmaceutical tablets was developed and proposed to be validated and applied. The analysis method was highly precise (presenting the relative standard deviation values within the accepted range of values, $RSD \leq 5\%$), and highly accurate (presenting the average recovery and average relative error value located within the accepted range of values, $Xd_m \leq 5$).*

Key-words: spectrophotometric method, validation, precision, accuracy

Rezumat. *O metodă spectrofotometrică de analiză în domeniul vizibil a conținutului de compus activ din comprimatele farmaceutice a fost propusă pentru validare şi aplicare. Metoda propusă a prezentat o precizie mare (valorile relative ale deviației standard fiind încadrate în limita de variație recomandată, $RSD \leq 5\%$) şi o acuratețe ridicată (valorile erorii relative fiind încadrate în limita de variație recomandată, $Xdm \leq 5$).*

Cuvinte cheie: metoda spectrofotometrică, validare, precizie, acuratețe

INTRODUCTION

To find out the active compound content of pharmaceutical tablets, a spectrophotometric method in visible range was developed and proposed to be validated and applied (Dorneanu *et al.,* 2003; Dorneanu *et al.,* 2007). Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results, being an integral part of any good analytical practice (ISO/IEC 17025).

One of the method's validation stage involved precision and accuracy evaluation. Precision of the method being a description of random errors, a measure of statistical variability, it was investigated under two aspects:

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repeatability and reproducibility (Roman et al., 1998; Boiculesii *et al.,* 2007; Tulasamma *et al.,* 2016; Aboud *et al.,* 2017, Singh *et al.,* 2015). Repeatability is expressing the consistency of the measurements under identical experimental conditions at short time intervals (in the same day), while reproducibility is expressing the fidelity of the measurement at large intervals of time (in different days). Accuracy is a description of systematic errors, a measure of statistical bias, that cause a difference between the obtained result and the true value. Accuracy of a method may be determined, by calculating a relative error, which is expressing a close correlation between a true, reference value and the analytical result of the laboratory measurements.

MATERIAL AND METHOD

Investigation of reproducibility consisted in carrying out the analysis on many samples from the same set of standard solutions in different days under the same given conditions (intermediate precision). Repeatability measurements consisted in conducting the analysis made on many samples coming from the same set of standard solutions in the same day under the same conditions (Bhalani *et al.,* 2015; Banjare *et al.,* 2013). Standard deviation (SD) and relative standard deviation (RSD %) were calculated for the tests carried out in the same day (intra-day test) and in different days (inter-day test).

To investigate the precision of the method, three solutions with different concentrations of analyte (2 μg/mL, 10 μg/mL and 25 μg/mL), were processed with the same reagents under the same conditions. Absorbances were measured at the wavelength λ = 690 nm. Four separate determinations have been made for each solution, three times in the same day (intra-day precision) and four determinations have been made in three different days (inter-day precision) by recording the mean absorbance's values.

Concentration C_C (μg/mL) was determined from the regression equation line: $A = 0.0234\ C_C (\mu g/mL) - 0.0031$, thus:

$$C_C (\mu g/mL) = (A + 0.0031) / 0.0234 \qquad (1)$$

Recovery (%) was determined with the formula (Roman *et al.,* 1998; Boiculesii *et al.,* 2007]:

$$\text{Recovery (\%)} = (C_C (\mu g/mL) \times 100) / C_T (\mu g/mL) \qquad (2)$$

For accurate measurements, recovery R (%) should be in the following range: 85% ≤ R % ≤ 105%.

Standard deviation (SD) was calculated with STDEV function in Microsoft Office Excel 2016 while relative standard deviation (RSD %) was determined with equation (3):

$$\text{RSD \%} = (SD \times 100) / R_{average} \qquad (3)$$

whereas $R_{average}$ was average recovery (%) value.

Thus, method precision was expressed as relative standard deviation (RSD %) of the calculated recovery R (%) values. For appropriate measurements, RSD≤ 5% (Banjare *et al.,* 2013; Mubeen *et al.,* 2009).

To determine method accuracy, three solutions of various concentrations (2 μg/mL, 10 μg/mL and 25 μg/mL) were selected to interact under the same experimental conditions. Four investigations for each solution were achieved in different moments of the same day and resulted absorbances were measured for λ = 690 nm. Then, the average absorbances, the recovery (%), the minimum and maximum values were calculated (Dorneanu *et al.,* 2003; Dorneanu *et al.,* 2007, Roman *et al.,* 1998; Boiculesii *et al.,* 2007; Tulasamma *et al.,* 2016; Aboud *et al.,* 2017, Singh et *al.,* 2015). Recovery (%) values were established by using equation (2). Standard deviation (SD) and relative standard deviation (RSD %) were calculated by using equation (3).

Accuracy was determined, by calculating a relative error Xd, which is expressing a close correlation between a true, reference value (Xa) and the analytical result of the laboratory measurements (Xr). Relative error X_D was calculated for the same three sample concentrations (2 µg/mL, 10 µg/mL and 25 µg/mL), according to formula:

$$\mathbf{Xd} = \frac{|\mathbf{Xr} - \mathbf{Xa}|}{\mathbf{Xa}} \cdot \mathbf{100} \le \mathbf{5\%} \qquad (4)$$

Whereas Xr is the measured value (calculated concentration), Xa is the real/true value (theoretical concentration) and Xd is the relative error.

RESULTS AND DISCUSSIONS

Measured absorbances of all three standard solutions, their theoretical concentrations (C_T), their calculated concentrations (C_C) expressed in µg/mL and their recovery (%) values are presented in table 1 and table 2.

Table 1

Intra-day precision test of the spectrophotometric method

Theoretical concentration C_T (µg/mL)	Mean absorbance (A_m)	Calculated concentration (C_C) (µg/mL)	Recovery (%)	Standard Deviation (SD)	RSD (%)
2.0	0.0429	1.966	98.30	1.660	1.629
	0.0429	1.966	98.30		
	0.0431	1.974	98.70		
	0.0428	1.962	98.10		
10.0	0.2360	10.218	102.18		
	0.2357	10.205	102.05		
	0.2358	10.209	102.09		
	0.2357	10.205	102.05		
25.0	0.5800	24.918	99.67		
	0.5795	24.897	99.58		
	0.5795	24.897	99.58		
	0.5798	24.910	99.64		

For the intra-day precision test, the average recovery (%) value was $R_{average}$ = 100.020%, while the standard deviation was SD = 1.660 (table 1). According to equation (3), the relative standard deviation (RSD = 1.629 %) was situated within normal limits range, being ≤ 5%.

For the inter-day precision testing of the spectrophotometric method, the average recovery (%) was $R_{average}$ = 100.414 %, while the standard deviation was

SD = 1.395. The relative standard deviation (RSD = 1.389%) was within normal limits, being ≤ 5%.

Table 2

Inter-day precision test of the spectrophotometric method

Theoretical concentration C_T (µg/mL)	Mean absorbance (A_m)	Calculated concentration C_C (µg/mL)	Recovery (%)	Standard Deviation (SD)	RSD (%)
2.0	0.0432	1.979	98.95	1.395	1.389
	0.0433	1.983	99.15		
	0.0433	1.983	99.15		
	0.0435	1.991	99.55		
10.0	0.2361	10.222	102.22		
	0.2361	10.222	102.22		
	0.2363	10.231	102.31		
	0.2363	10.231	102.31		
25.0	0.5804	24.936	99.74		
	0.5804	24.936	99.74		
	0.5807	24.949	99.80		
	0.5809	24.957	99.83		

The evaluation results of the system precision, done for 4 µg/mL standard solution, are presented in table 3. The absorbances corresponding to standard solution presented close, one to another, values.

The relative standard deviation (RSD = 0.235%) was within the normal range of values.

Table 3

System precision

Det. No.	Mean aborbance (A_m) values
1.	0.0920
2.	0.0924
3.	0.0925
4.	0.0925
5.	0.0922
Mean	0.092320
SD	0.000217
RSD (%)	0.235

In order to evaluate the accuracy of the method, absorbance values, calculated concentrations (C_C) and recovery (%) values were determined (table 4).

Table 4

Accuracy of the method

Theoretical concentration C_T (µg/mL)	Average absorbance (A)	Calculated concentration C_C (µg/mL)	Recovery (%)
2.0	0.0428	1.962	98.10
	0.0428	1.962	98.10
	0.0430	1.970	98.50
	0.0429	1.966	98.30
10.0	0.2357	10.205	102.05
	0.2358	10.209	102.09
	0.2356	10.201	102.01
	0.2355	10.197	101.97
25.0	0.5795	24.897	99.58
	0.5795	24.897	99.58
	0.5798	24.910	99.64
	0.5797	24.906	99.62

Average recovery was $R_{average}$ = 99.962 %, while the standard deviation was SD = 1.6365. According to equation (3), the relative standard deviation value (RSD = 1.6376 %) was located within normal range of values. Minimum recovery (%) calculated value was 98.10 % while the maximum recovery value was 102.09 % (table 4).

The relative error (Xd%) was calculated for each of the three solution concentrations (table 5).

Table 5

Calculated relative error values (Xd %)

C_T (µg/mL)	Xd (%)
2.0	1.900
	1.900
	1.500
	1,700
10.0	2.050
	2.090
	2.010
	1.970
25.0	0.412
	0.412
	0.360
	0.376

Individual values of the relative error (table 5) were below 5%. Also, the average relative error (Xd_m = 1.390 %) was within the normal range of values, being ≤ 5 %.

CONCLUSIONS

The spectrophotometric analysis method was highly precise presenting the relative standard deviation values (RSD = 1.629 % for intra-day precision test and RSD = 1.389 % for inter-day precision test) located within the accepted range of values (RSD ≤ 5%).

The spectrophotometric analysis method was highly accurate presenting the average recovery ($R_{average}$ = 99.962 %) and average relative error value (Xd_m = 1.390 %) located within the accepted range of values ($Xd_m \leq 5$).

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