USE OF DATAEXPLORER ONLINE FOR DATA PROCESSING IN THE DETERMINATION OF ACTIVE COMPONENTS OF DRUG

Maria M. Savanović[1,2],
[0000-0003-3907-6259]

Milinko Perić[3],
[0000-0001-8429-0946]

Andrijana Bilić[1,2],
[0000-0002-2912-7318]

Stevan Armaković[2,4],
[0000-0002-8049-9969]

Sanja J. Armaković[1,2*]
[0000-0002-3665-1046]

[1]University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection
Novi Sad, Serbia

[2]Association for the International Development of Academic and Scientific Collaboration,
Novi Sad, Serbia

[3]University of Novi Sad, BioSense Institute,
Novi Sad, Serbia

[4]University of Novi Sad, Faculty of Sciences, Department of Physics,
Novi Sad, Serbia

Correspondence:
Sanja J. Armaković

e-mail:
sanja.armakovic@dh.uns.ac.rs

Abstract:

This paper aimed to demonstrate the use of the newly developed DataExplorer Online software for statistical analysis to evaluate the performance parameters of two different analytical techniques. Namely, HPLC–DAD (High Performance Liquid Chromatography Diode Array Detection) and spectrophotometry were used to measure the concentration of metoprolol in commercial drug tablets. The tools used for methods performance evaluation in the analysis of metoprolol were DataExplrorer Online. The online software tool DataExplorer (available at https://dataexplorer.online) was developed to support the analysis of large amounts of data and the validation of analytical methods. In addition, DataExplorer Online enables the assessment of normal distribution, which is unavoidable before ANOVA analysis. Depending on the number of examined factors, it offers One, Two, Three, and Multivariate ANOVA analysis. Therefore, DataExprorer Online is suitable for specific needs that may arise in different experimental studies. Statistical analysis in DataExprorer Online, which included checking for normal distribution and applying One way ANOVA, indicated that HPLC–DAD and spectrophotometric methods are suitable for routine analysis of metoprolol in drug tablets. Overall, results showed that HPLC–DAD and spectrophotometry contribute to precise and accurate analytical processes.

Keywords:

Metoprolol, HPLC, Spectrophotometry, ANOVA, Data Science.

INTRODUCTION

In the past years, many procedures for the detection of drug-active components have evolved using different analytical techniques [1]. From an analytical perspective, finding a suitable, green method that can enable the determination of low drug concentrations is challenging and complex [2]. Metoprolol tartrate, a widely used selective β1-blocker [3], is commonly prescribed for various cardiovascular disorders[4], including hypertension, angina pectoris, and as prophylaxis post-myocardial infarction [5]. The drug has poor bioavailability due to its high first pass metabolism [6]. Commercial formulations are usually prescribed twice a day. This drug tends to lose its action of selectivity at higher plasma concentrations [7]. Because of its frequent use, it is necessary to apply reliable analytical techniques in the analysis of metoprolol, also considering their environmental impact, in order to provide reliable analytical results [8].

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Drug analysis includes experiments for measuring the drug concentration as an active drug component in a commercial formulation/tablet [9]. Such measurements could be performed with a suitable analytical method [10]. A wide span of spectroscopic and chromatographic techniques, alone or as hybrid adjustments, have been applied for many years in the analysis of drugs [11]. In the process of drug analysis, liquid chromatographic techniques have become the baseline for the determination of drugs and, as such, have gained supreme importance [12]. They are followed by the separation of drug-active components from the other chemical entities that are interfering, which could be intermediates or impurities [13]. High performance liquid chromatography (HPLC), high performance thin layer liquid chromatography (HPTLC), and combined hybrid versions are omnipresent and unique chromatographic techniques that have a leading position in drug analysis [14]. However, there are still some challenges regarding these techniques [15]. When commercial formulations are available without combinations of many drugs, or the drug-active components are isolated during sample preparation, it is then suitable to apply an analytical method that is simple, rapid, accurate, and sensitive, such as the spectrophotometric technique [16]. The UV–Vis spectrophotometric technique can be applicable to the analysis of various drug-active components because of its simplicity [17], the availability of the spectrophotometer, and low price [18]. The spectrophotometric method often serves as an alternative method to the methods described in pharmacopeias [19]. Demonstrating the effectiveness of the mentioned techniques can best be realized and used with a primary focus on the steps associated with them, as well as the analyte samples preparation [20]. Further, it is necessary to apply appropriate statistical analysis of the obtained data [21].

Estimating analytical methods performance is an essential task to obtain reliable results that can be further processed [22]. For method application, besides being reliable, it is also essential that the analytical procedure is environmentally friendly and safe for humans [23]. The amounts of generated waste, the toxicity of reagents, the number of procedural steps, energy requirements, automatization, and miniaturization are some of the criteria considered when assessing the greenness of the analytical method [24].

After analytical measurements, the data should be processed statistically in a proper way [25]. Statistical analysis of experimental results is essential to discuss [26].

In the evaluation of method performance, statistical analysis can show that methods are complementary rather than contradictory [27]. Also, statistical analysis of a data set cannot rescue a poorly designed study, which implies that a good experimental design is essential [28].

In this work, two different analytical techniques were used to analyze metoprolol isolated from commercial drug tablets. The results highlight the importance of choosing suitable analytical techniques in the analysis of drug active components. Namely, HPLC–DAD and spectrophotometric analytical techniques were compared in the study of metoprolol in commercial drug tablets. After obtaining data, the evaluation of analytical techniques performance was asses using the DataExplorer software tool. The normal distribution of the obtained results was verified using DataExplorer. Additionally, One-way ANOVA (Analysis of Variance), another feature in DataExplorer Online, was utilized.

2. METHODOLOGY

2.1. MATERIALS AND SAMPLE PREPARATION

The chemicals used are pro-*analysis* grade and used without further purification. Metoprolol (≥98 %, Sigma-Aldrich, M = 684.81 g/mol, $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$, CAS No 56392-17-7) was used as a standard solution. The sample was Presolol® tablets of 50 mg (Hemofarm, Serbia). For sample preparation, the tablet was dissolved in a 1.00 dm^3 measuring vessel in ultrapure water, shaken, and heated for 10 min at 36±1°C. After cooling, the vessel was filled with ultrapure water to the mark (submitted to the *Journal of the Indian Chemical Society*). Before measurements, the solutions were filtered using a membrane filter. Other used chemicals (mobile phase components for liquid chromatography) were 99.9% acetonitrile C_3H_3NO (Sigma-Aldrich, Germany) and 85% orthophosphoric acid, H_3PO_4 (Lachema, Czech Republic).

2.2. HPLC–DAD AND SPECTROPHOTOMETRIC MEASUREMENTS

Chromatography measurements were performed using HPLC–DAD (Shimadzu) with an Eclipse XDB–C18 column (150 mm · 4.6 mm, particle size 5 μm, 25 °C). The UV/vis DAD detector was adjusted at 223 nm (metoprolol absorption maximum).

The mobile phase flow rate was 0.8 cm^3/min. The mobile phase contained a mixture of acetonitrile and ultrapure water (0.1% H_3PO_4), with a gradient of: 0 min 15% acetonitrile, increased to 30% acetonitrile during the next 5 min, then 30% acetonitrile was constant for the next 5 min, post time was 2 min. Spectrophotometric measurements were conducted using a double-beam T80+UV/Vis Spectrometer (UK) at a slit width (2 nm). For the measurements, a quartz cell (1 cm optical length) and the computer-loaded UV Win 5 data software were used.

2.3. DATAEXPLORER ONLINE SOFTWARE TOOL

DataExplorer is a software tool developed for statistical data analysis. It is available at https://dataexplorer.online/. DataExplorer Online is a user-friendly and easily accessible online software tool that helps users understand data. Tools from DataExplorer Online that were used in this research were standard deviations, normal distribution, and ANOVA test.

3. RESULTS AND DISCUSSION

The concentration of metoprolol in 30 tablets of 50 mg was determined by employing the HPLC-DAD and spectrophotometric approach. The theoretical concentration of the active component in the tablet should be 0.073 mmol/dm^3. Therefore, the mean values ($\bar{x}$), and standard deviations (*SD*) were calculated using DataExplorer Online (Figure 1). Obtained results indicate that $\bar{x}$ obtained using HPLC were the same as the correct value, as noted by the tablet producer.

In the case of the spectrophotometric technique, the value was 0.072 mmol/dm^3. However, the obtained *SD* was the same. Since *SD* indicates how accurately the mean represents sample data [29], it could be concluded that both methods provided accurate results.

ANOVA is a standard statistical method in data analysis. However, for ANOVA to be applicable, the distribution of mean scores must follow a normal (Gaussian) distribution [30]. The meaning of the normal distribution is that when the values change, they tend to stay near the average point, so they are distributed around that average in a smooth, bell-shaped curve [31]. Therefore, we checked if the results obtained by HPLC and spectrophotometry are normally distributed. The normal distribution tool from DataExplorer Online was used for these calculations. The null hypothesis (H_0): Data follows the normal distribution. Results shown in Figure 2 suggested that the data follows a normal distribution (fail to reject H_0). The values of the Shapiro-Wilk Test additionally confirmed that: statistic = 0.98 and *p*-value = 0.25. Since $p>0.05$, H_0 failed to reject, and data follows a normal distribution. Since data follows a normal distribution, further data analysis could be performed using ANOVA.

ANOVA is variance analysis [32], and the DataExplorer Online software tool offers One way ANOVA, Two way ANOVA, Three way ANOVA, and Multivariate ANOVA (MANOVA) (Figure 3). Since when using one way ANOVA, the means of two or more groups for one dependent variable are compared, this tool was chosen for use in DataExplorer Online. The dependent variable was the analytical technique, and the levels were HPLC and spectrophotometry.

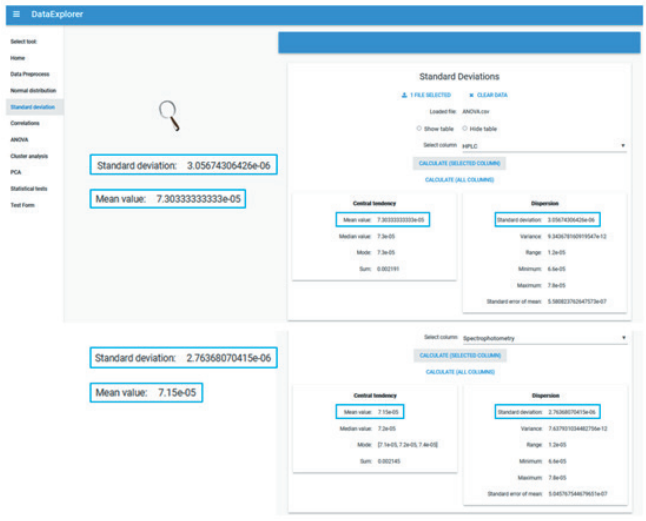

Figure 1. Uploading the input file for $\bar{x}$ and *SD* analysis using DataExplorer Online.

H_0: The statistically significant difference was not present in variation between groups, with the variation within groups. The maximum allowed error range, which can indicate that differences in means exist, can be defined as significance level [32]. *p*-value was used to decide whether to reject the H_0. The convention in many scientific fields is to reject the H_0 if the *p*-value is less than 0.05, often referred to as the level of significance [33]. One way ANOVA was applied to analyze results obtained by HPLC and spectrophotometry for metoprolol in order to observe differences in the amount of variation between groups, with the variation within groups. Obtained outcomes were $F = 4.15$ and $p = 0.05$ (Figure 3).

Interestingly, *the* *p*-value was exactly 0.05, which means that it is located right on the limit of whether the difference in variation between groups and within groups exists. Based on the literature data [34], it can be stated that a statistically significant difference was not present in variation between groups and within groups. More precisely, a statistically significant difference was not present in the results obtained for metoprolol using HPLC and spectrophotometric technique.

4. CONCLUSION

The purpose of the work was to highlight the importance of selecting suitable analytical techniques in the analysis of drug-active components. The performance of HPLC–DAD and spectrophotometric techniques was evaluated in the study of metoprolol. This was achieved using the DataExplorer Online software tool. Obtained data from HPLC–DAD and spectrophotometric measurements were further subjected to data processing through tools available in DataExplorer. First, the mean values, as well as standard deviations, were calculated using DataExplorer. The obtained results indicated that both methods provided accurate results since SD was 0.003 mmol/dm^3 for both methods. Through DataExplorer, normal distribution was checked, and obtained graphs showed that results were normally distributed. Further, results were analyzed using One way ANOVA since the dependent variable was analytical technique and the levels were HPLC and spectrophotometry. A *p*-value of 0.05 indicated that a statistically significant difference was not present between HPLC–DAD and the spectrophotometric method in the analysis of metoprolol.

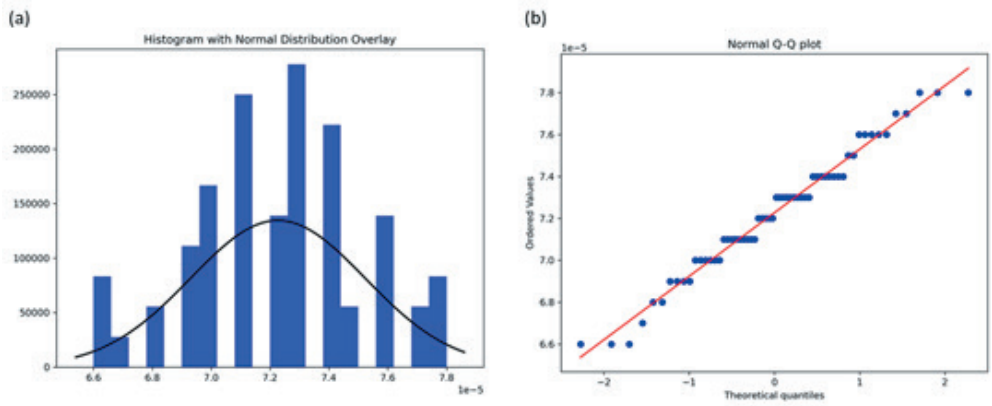

Figure 2. Histogram with normal distribution overlay and (b) normal Q-Q plot for the results obtained by HPLC and spectrophotometric technique.

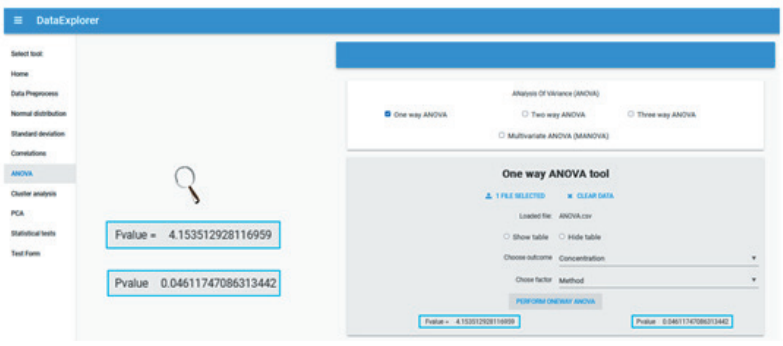

Figure 3. Uploading the input file for ANOVA analysis using DataExplorer Online.

The study concluded that both HPLC–DAD and spectrophotometry contribute to precise and accurate analytical processes.

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