ORIGINAL ARTICLE / ÖZGÜN MAKALE

ZERO-ORDER AND FIRST-DERIVATIVE SPECTROPHOTOMETRY FOR THE DETERMINATION OF SPIRONOLACTONE IN PHARMACEUTICAL TABLETS

FARMASÖTİK TABLETLERDEKİ SPİRONOLAKTONUN TAYİNİ İÇİN SIFIR DERECEDEN VE BİRİNCİ TÜREV SPEKTROFOTOMETRİ

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ABSTRACT

Objective: *Spironolactone, a potassium-sparing diuretic drug on the WHO's Essential Medicines List, is primarily used to treat fluid retention resulting from heart failure, kidney disease, and liver scarring. In this research article, zero-order, and first-derivative spectrophotometric approaches were applied to the quantity of spironolactone in pharmaceutical tablet formulation without any separation procedure.*

Material and Method: *Spironolactone compound was obtained from Sigma-Aldrich (Steinheim, Germany). As a solvent methanol (J.T. Baker, Netherlands, HPLC grade) was used for UV-visible spectrophotometric assays. In the implementation of the zero-order and first-derivative approaches, the quantification of Spironolactone was carried out using UV spectrophotometric measurements in the 200-300 nm wavelength range (with a 2 nm increment) and 1 cm quartz cells. These approaches were tested by analyzing spironolactone in independent test samples and spiked samples. The tablet analyses of spironolactone were performed on ALDACTONE®-A 25 mg Tablet supplied by Aris Ali Raif Pharmaceuticals Industry, Başakşehir, İstanbul, Türkiye.*

Result and Discussion: *The analysis of spironolactone was conducted using zero-order values measured at 239 nm and first-derivative values measured at 250.4 nm (n=3). The calibration graphs created at these wavelengths showed linearity within the 6.0 to 20.0 µg/ml concentration range, with correlation coefficients of r = 0.9996 for the zero-order method and r = 0.9997 for the first-order derivative method. The validity of the methods was proved by analyzing spironolactone in independent test samples and spiked samples. These methods were then applied to the quantitation of spironolactone in commercial tablets and a good agreement was reported between label claim assay results.*

Keywords: *Antihyypertensive drug, determination of spironolactone, first derivative spectrophotometry, tablet analysis, zero-order spectrophotometry*

ÖZ

Amaç: *WHO'nun Temel İlaçlar Listesi'nde yer alan potasyum tutucu bir diüretik ilaç olan spironolakton, öncelikli olarak kalp yetmezliği, böbrek hastalığı ve karaciğer skarlaşmasından*

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kaynaklanan sıvı tutulumunu tedavi etmek için kullanılır. Bu araştırma makalesinde, herhangi bir ayırma prosedürü olmaksızın farmasötik tablet formülasyonundaki spironolakton miktarına sıfırıncı mertebeden ve birinci türev spektrofotometrik yaklaşımlar uygulandı.

Gereç ve Yöntem: *Spironolakton bileşiği Sigma-Aldrich'ten (Steinheim, Almanya) elde edildi. Çözücü olarak metanol (J.T. Baker, Hollanda, HPLC sınıfı) UV-görünür spektrofotometrik analizler için kullanıldı. Sıfırıncı mertebe ve birinci türev yaklaşımlarının uygulanmasında, Spironolaktonun kantifikasyonu 200-300 nm dalga boyu aralığında (2 nm artışla) ve 1 cm kuvars hücrelerde UV spektrofotometrik ölçümler kullanılarak gerçekleştirildi. Bu yaklaşımlar, spironolaktonun bağımsız test örneklerinde ve spike edilmiş örneklerde analiz edilmesiyle test edildi. Spironolaktonun tablet analizleri, Aris Ali Raif İlaç Sanayi, Başakşehir, İstanbul, Türkiye tarafından tedarik edilen ALDACTONE®-A 25 mg Tablet üzerinde gerçekleştirildi.*

Sonuç ve Tartışma: *Spironolakton analizi, 239 nm'de ölçülen sıfırıncı mertebeden değerler ve 250,4 nm'de ölçülen birinci türev değerleri kullanılarak gerçekleştirildi (n=3). Bu dalga boylarında oluşturulan kalibrasyon grafikleri, sıfırıncı mertebeden yöntem için r = 0,9996 ve birinci mertebeden türev yöntemi için r = 0,9997 korelasyon katsayılarıyla 6,0 ila 20,0 µg/ml konsantrasyon aralığında doğrusallık gösterdi. Yöntemlerin geçerliliği, bağımsız test örneklerinde ve güçlendirilmiş örneklerde spironolaktonun analiz edilmesiyle kanıtlandı. Daha sonra bu yöntemler ticari tabletlerdeki spironolaktonun kantifikasyonuna uygulandı ve etiket iddiası deney sonuçları arasında iyi bir uyum bildirildi.*

Anahtar Kelimeler: *Antihipertansif ilaç, birinci türev spektrofotometri, sıfırıncı mertebe spektrofotometri, spironolakton tayini, tablet analizi*

INTRODUCTION

Cardiovascular diseases, which are among the most common chronic and non-communicable diseases in the world, are diseases with high social and economic costs that can result in organ damage, stroke, and even death if left untreated [1-5]. The initial pharmacological treatment for hypertension includes calcium channel blockers, thiazide diuretics, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers, or a combination of two of these agents [6-7]. Spironolactone, a potassium-sparing diuretic drug on the WHO's Essential Medicines List, is primarily used to treat fluid retention resulting from heart failure, kidney disease, and liver scarring [8-9]. In addition, it is also used in the treatment of precocious puberty (for boys), excessive hirsutism, and acne (for women), and as part of transgender hormone therapy (for transgender women) [10-12]. The chemical name of spironolactone, whose chemical structure is given in Figure 1, is 7α-acetylthio-3-oxo-17α-pregn-4-ene 21,17β-carbolactone.
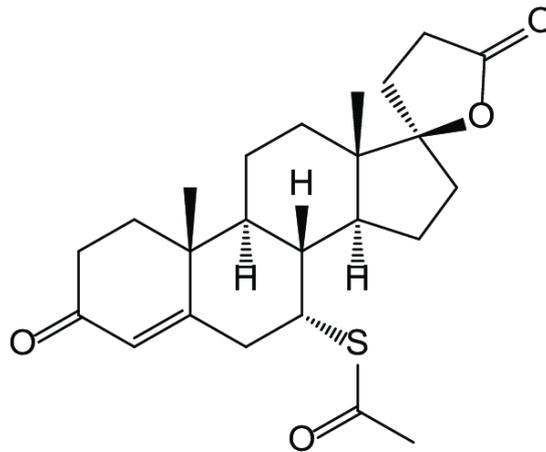

Figure 1. Chemical construction of spironolactone

The literature review stated various analytical methods for determining spironolactone alone or in combination with other active pharmaceutical ingredients, including chromatographic [13-17],

voltammetric [18-20], and spectrophotometric [21-25] techniques. This indicates that there are no reported analytical methods for quantitatively estimating spironolactone using zero-order and first-derivative spectrophotometry. Due to the possibility of derivatizing zero-order spectra by computer programs, there is a need for more accurate and sensitive derivative spectrophotometry, which allows valuable analytical treatments. Derivative spectrophotometry eliminates excipients' effect in commercial products, matrix effects from sample turbidity, and noise peaks from instruments and provides an adequate resolution for complex systems when the analysis conditions are suitable [26].

First-derivative spectrophotometry calculates and plots a derivative of a spectral curve, providing an alternative approach for pharmaceutical analysis. Therefore, this article aimed to develop and validate a new, straightforward, rapid, accurate, sensitive, and precise zero-order and first derivative spectrophotometric approach for the quality control and routine analysis of spironolactone in pharmaceutical tablet form.

MATERIAL AND METHOD

Apparatus and Software

UV visible spectrophotometric records were gathered using a Shimadzu double-beam UV-Visible spectrophotometer (UV-2550, Kyoto, Japan) featuring a fixed slit width (2 nm). Absorbance measurements were taken using a 1 cm quartz cuvette, in the wavelength range of 200-300 nm, and at 0.2 nm intervals. After transferring the data to Microsoft Excel (Microsoft, USA), it was used for processing the spectra and conducting statistical and regression analysis. Additionally, all graphical representations were generated.

Chemicals and Reagents

Spironolactone compound was obtained from Sigma-Aldrich (Steinheim, Germany). As a solvent methanol (J.T. Baker, Netherlands, HPLC grade) was used for UV-visible spectrophotometric assays. Working solutions were freshly and daily prepared and stored in the dark and the refrigerator throughout the analysis.

Preparation of Standard, Calibration, and Validation Solutions

A stock solution of spironolactone was prepared by accurately weighing 10.0 mg of the compound and dissolving it in methanol. The resulting solution was quantitatively transferred to a 100 ml volumetric flask and diluted to volume with methanol to obtain the final stock solution. This stock solution was subsequently utilized to prepare both standard solutions for calibration and independent validation solutions. For the preparation of standard solutions, a series of dilutions were performed to achieve concentrations in the range of 6.0 to 20.0 µg/ml. These solutions were used to establish calibration curves, employing both the zero-order absorption and first-derivative spectrophotometric methods. Calibration curves were constructed by plotting absorbance against concentration for each method. To ensure the accuracy and linearity of the calibration, multiple concentration levels were included in this range. For method validation, independent test samples were prepared at specific concentrations of 6.0, 14.0, and 20.0 µg/ml following the same procedure as used for the calibration standards. These validation solutions were subjected to analysis using the same spectrophotometric methods to verify the precision, accuracy, and reproducibility of the analytical method.

In the standard addition method, known quantities of spironolactone were spiked into a sample of the pharmaceutical tablet solution to account for any potential matrix effects. Specifically, spironolactone standard solutions at four different concentration levels (0, 4, 8, and 12 µg/ml) were added to 0.6 ml of the pharmaceutical tablet solution, which was then diluted to 10 ml in a volumetric flask. This procedure was repeated for each concentration level, and all experiments—including the preparation of calibration standards, validation samples, and pharmaceutical tablet solutions—were conducted in triplicate to ensure consistency, precision, and reliability of the results.

By conducting the experiments in triplicate, statistical validation of the method was ensured, providing confidence in both the repeatability and robustness of the spectrophotometric determination

of spironolactone in pharmaceutical formulations.

Preparation of Tablet Samples

In the implementation of the zero-order spectrophotometric and first-derivative spectrophotometric methods in commercial pharmaceutical tablet analysis, 10 tablets of ALDACTONE®-A 25 mg Tablet (Aris Ali Raif İlaç Sanayi, Başakşehir, İstanbul, Türkiye) were weighed precisely and ground into powder in a mortar. After determining the amount equivalent to one-tenth of a tablet, it was dissolved in methanol in a 25 ml volumetric flask. The solution was mixed using a mechanical stirrer for 30 minutes and then filtered through a 0.22 µm pore size PVDF filter (Isolab, Wertheim, Germany). For analysis, 1.8 ml of the filtered solution was transferred to a 10 ml volumetric flask, and the volume was made up to 10 ml with the same solvent.

RESULT AND DISCUSSION

When spectrophotometric methods are compared with other methods such as chromatography and electrophoresis, the widespread availability of instrumentation, the rapidity and simplicity of the procedures, and the high sensitivity and accuracy of the methods still make spectrophotometric methods attractive. In spectrophotometric methods, various techniques are applied to obtain qualitative and quantitative information from spectra consisting of unresolved bands. The most important of these is derivative spectrophotometry, which is obtained by applying the first or higher-order mathematical derivative ($dA/d\lambda$) of wavelength versus absorbance curves (zero-order spectra). This technique generally improves the resolution of bands, removes the influence of the matrix, and supplies more characterized fingerprints than direct absorbance spectra (zero absorption spectra), since it increases the detectability of minor spectral properties. In this context, we applied first derivative spectrophotometry to zero-order absorption spectra to directly quantify spironolactone in the pharmaceutical preparation. For this purpose, the suitable calibration range of spironolactone, complying with Beer's law, was determined as 6.0-20.0 µg/ml. In this calibration range, zero-order spectra were recorded in the wavelength range of 200-300 nm and the wavelength at which spironolactone gave maximum absorption was determined as 239 nm. The obtained spectra are presented in Figure 2.
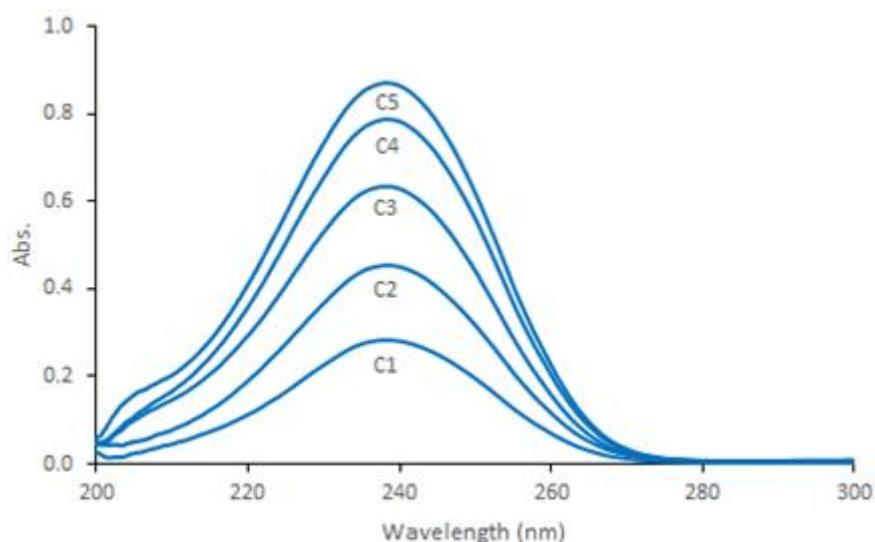

Figure 2. Zero-order spectra of spironolactone in the 6.0-20.0 µg/ml calibration range

The first derivative spectra of spironolactone were plotted with $\Delta\lambda = 2$ nm intervals and a scaling factor of 30 from the computed zero absorption spectra of the calibration samples obtained by measuring the $dA/d\lambda$ value at 250.4 nm as in Figure 3.
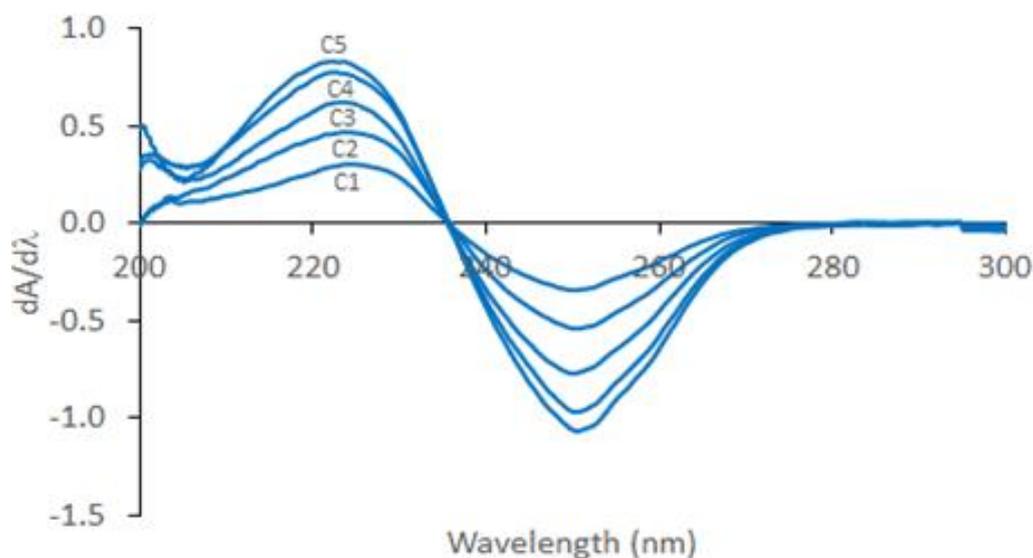
Figure 3. The first-derivative spectra of spironolactone in the 6.0-20.0 µg/ml calibration range

The calibration curves for spironolactone were obtained by measuring absorbances at the selected wavelength of 239 nm for zero-order spectra and 250.4 nm for first-derivative spectra, respectively. The statistical regression analysis results are given in Table 1. Then, spironolactone was estimated through the obtained calibration curves.

Table 1. Statistical overview: Results of the linear regression analysis

Parameter	Zero-order	First derivative
m	0.0417	0.0515
n	0.0382	0.0381
r	0.9996	0.9997
SE(m)	7.13×10^{-4}	7.85×10^{-4}
SE(n)	1.04×10^{-2}	1.14×10^{-2}
SE(r)	8.17×10^{-3}	7.85×10^{-4}
LOD (µg/ml)	0.75	0.66
LOQ (µg/ml)	2.48	2.22

m, n, and r are the regression equation's slope, intercept, and correlation coefficients. SE (m), SE (n), and SE (r) are the standard errors of slope, intercept, and correlation coefficient, respectively. LOD: Detection limit (µg/ml), LOQ: Quantification limit (µg/ml)

Zero-order spectrophotometric and first-derivative spectrophotometric methods were validated by analyzing the independent test samples and spiked samples (prepared by adding the standards to the pharmaceutical tablet solution). Validation parameters such as range, linearity, accuracy, precision, selectivity, the limit of detection (LOD), and the limit of quantitation (LOQ) were given in Table 1. Correlation coefficients higher than 0.9990 were obtained for zero-order and first-derivative methods. Limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated using the calibration curves' standard deviation and slope values , and the results are presented in Table 1. The validity, precision, accuracy, and reproducibility of two different proposed methods were verified by analyzing independent test samples of spironolactone. The average of the three results, mean recovery, and relative standard deviation values are given in Table 2. As can be seen in this table, these methods achieved desirable precision and accuracy without requiring any preliminary separation steps for the analysis of spironolactone.

Table 2. Research on recovery and the outcomes achieved by applying methods to independent test samples

Added	Found* (µg/ml)		Recovery (%)	
(µg/ml)	Zero-order	First-derivative	Zero-order	First-derivative
	239 nm	250.4 nm		
6	5.85	5.94	97.5	99.0
14	14.11	14.06	100.8	100.4
20	19.98	19.99	99.9	99.9
		Mean	99.40	99.77
		Standard Deviation	1.71	0.70
		Relative Standard Deviation	1.72	0.71

* Results were obtained by averaging the results of three assays for each concentration level

To show whether the excipients in the tablet affect the determination of the spironolactone, spiked samples were prepared at 4, 8, and 12 µg/ml concentration levels and analyzed with the proposed zero-order and first-derivative methods. Then added recovery results for each drug were calculated. Their results (recovery value and standard deviation) are given in Table 3. The results of the experiments were calculated as the average of triplicates for each concentration level. As can be seen from the results in Table 3, it was observed that the sample matrix did not affect the analysis of spironolactone. In other words, the results were found to be satisfactory in terms of the selectivity of the proposed analytical methods.

Table 3. Recovery results obtained by implementing the methods in spiked samples

Added (µg/ml)	**Tablet**	**6**	**6**	**6**
	Standard	4.00	8.00	12.00
Found (µg/ml)	Zero-order	3.98	8.18	12.09
	First derivative	3.86	8.10	12.03
Recovery (%)	Zero-order	99.50	102.20	100.70
	First derivative	96.50	101.20	100.20
RSD (%)	Zero-order	0.93	2.25	1.13
	First derivative	0.82	2.64	1.01

RSD: Relative standard deviation
The added amount of the tablet is approximately 6 µg/ml

Zero-order spectrophotometric and first-derivative spectrophotometric methods were used for the quantity of pharmaceutical tablet formulations containing spironolactone. Table 4 shows the assay results of spironolactone in commercial tablets. The results provided a good agreement with the reported label claim of the commercial sample. The standard deviation and relative standard deviation given in Table 4 confirm the applicability and performance of the proposed methods.

A statistical analysis was conducted on the experimental results obtained from using the zero-order and first-derivative methods to determine the concentration of spironolactone in a commercial tablet. This analysis was carried out using Microsoft Excel software. A paired t-test was performed at a significance level of $p = 0.05$ to compare the assay results of both methods. The statistical results indicated no significant difference between the outcomes of the zero-order and first-derivative methods. Therefore, it was concluded that both proposed methods yield comparable results, as demonstrated in Table 4.

Table 4. Quantitation results of spironolactone in tablet samples using zero-order and first-derivative methods

Experiment Number	mg/ tablet	
	Zero-order	First derivative
1	24.87	24.91
2	25.09	25.23
3	24.65	24.66
4	24.73	24.83
5	24.77	24.97
Mean	24.82	24.92
Standard deviation	0.17	0.21
Relative standard deviation	0.68	0.83
	t-calculated= 2.306	t-tabulated=2.571
	p=0.438	p=0.05

Label amount: 25 mg spironolactone per tablet

Conclusion

In analytical procedures, it is crucial to quantify compounds in matrix systems without any separation processes. As it is known, methods such as chromatography and capillary electrophoresis, which use separation techniques that require expensive and complex devices, are tedious due to the difficulties in optimizing experimental conditions in the analysis processes, long analysis times, and in some cases not yield successful results, forcing analytical chemists to search for faster, more easily applicable and more economical methods. In this research article, zero-order and first derivative methods were successfully implemented for the assay of spironolactone from pharmaceutic preparations because they are easier to apply, faster and more economical, and provide reproducible, sensitive, accurate, and reliable results. It can be easily used for routine analysis of spironolactone in scientific studies and industry.

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AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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