https://doi.org/10.52645/MJHS.2024.4.06

UDC: 615.254.1.012/.014:543.422.3



RESEARCH ARTICLE



# Analysis of spironolactone in compound powder by ultraviolet-visible spectrophotometry

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## ABSTRACT

**Introduction.** Spironolactone (Spir) is a selective and competitive antagonist of aldosterone that increases the excretion of water and sodium while decreasing the excretion of potassium (K+ sparing diuretic). The substance was studied to develop qualitative and quantitative methods of analysis and to validate them according to documents regulating the quality of active pharmaceutical ingredients in the development of pharmaceutical forms.

**Material and methods.** A new dosage form (powder) with Spir was developed and analyzed by a spectrophotometry method using a UV-Vis spectrophotometer (Agilent 8453, USA) with 10.0 mm matched quartz cells at 238±2nm, with methanol as the blank. The method was validated for specificity, linearity, precision, accuracy, robustness, LOD and LOQ.

**Results.** The method was found to be linear in the drug concentration range of 5.0 to 30.0 µg/ml, with a correlation coefficient ($R^2$) of 0.9994 for Spir. The LOD of Spir was 0.5 µg/ml and the LOQ was 1.4 µg/ml, indicating the method's sensitivity. The method was established as accurate (mean recovery values of concentration at 80%, 100%, 120% ranging between 99.9 and 101.7%). Repeatability precision and intermediate precision %RSD values amongst six sample solutions were from 0.13% to 0.25% for Spir (less than 2%). The accuracy (recovery) ranged between 99.9% and 101.7%, with standard deviations ranging from 0.08% to 0.17%.

**Conclusions.** In the presence of common excipients, such as microcrystalline cellulose, lactose monohydrate, and stearic acid, no interferences were observed. This method was found to be suitable for the routine analysis of Spir from the newly developed pharmaceutical form.

**Keywords:** spironolactone, UV-Vis spectrophotometry, validation.

**Cite this article:** Mazur E. Analysis of spironolactone in compound powder by ultraviolet-visible spectrophotometry. Mold J Health Sci. 2024;11(4):38-43. https://doi.org/10.52645/MJHS.2024.4.06.

**Manuscript received:** 10.05.2024

**Accepted for publication:** 15.09.2024

**Published:** 10.12.2024

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## Key messages

### What is not yet known on the issue addressed in the submitted manuscript

A new pharmaceutical dosage form was developed with spironolactone and other active ingredients (potassium orotate, potassium and magnesium aspartate). Until now, no method for dosing spironolactone in such a combination has existed. An easy, accurate, and efficient assay method for the simultaneous determination of spironolactone in this pharmaceutical dosage form was elaborated, which can be applied for routine analysis.

### The research hypothesis

If the results obtained by this developed method comply with validation requirements (specificity, linearity, precision, accuracy, robustness), then it can be applied to routine quality control, quantitative analysis, and stress stability testing.

**The novelty added by the manuscript to the already published scientific literature**

Spironolactone is combined for the first time in the same pharmaceutical form with potassium aspartate, magnesium aspartate, and potassium orotate. Thus, the spectrophotometric dosing technique for spironolactone in this combination is developed for the first time. The results obtained indicate that the proposed method is suitable for the analysis of spironolactone in the newly developed pharmaceutical form in the presence of other active ingredients, with excellent recovery, precision, and linearity.

## Introduction

Spironolactone is a selective and competitive antagonist of aldosterone, due to its structural similarity to aldosterone. Chemically, spironolactone is 7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone (Figure 1). Spironolactone works by competing with aldosterone for interactions with aldosterone receptors in the collecting duct. This antagonistic effect increases the excretion of water and sodium while decreasing the excretion of potassium ($K^+$ sparing diuretic) [1]. Unfortunately, spironolactone works slowly, requiring several days to develop its pharmacologic action, and similarly, its effect diminishes slowly [1].

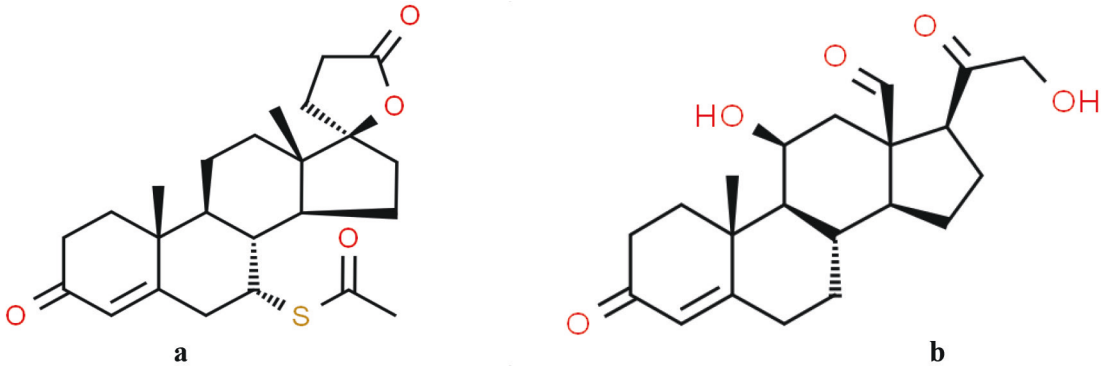


**Fig. 1** Chemical structure of spironolactone (a) and aldosterone (b) [2, 3].
Data from http://www.chemspider.com/Chemical-Structure.5628.html
Data from http://www.chemspider.com/Chemical-Structure.5633.html

To expand the portfolio of effective and harmless potassium-containing products with mechanisms of action at the molecular level, a new composition was developed, consisting of potassium orotate (OK), potassium and magnesium aspartate (AsMg, AsK). Excipients were included in the pharmaceutical form to improve technological parameters for facilitating the manufacturing process, as well as to enhance stability, bioavailability, and efficacy of the final medicine. Moreover, a validated method is necessary to analyze spironolactone in various dosage forms, such as suspension (5mg/ml), tablets (25mg), capsules (50mg), and the newly developed powder. The developed assay method was validated according to International Conference on Harmonization (ICH) Guidelines and was successfully employed for the analysis of spironolactone in pharmaceutical dosage forms, based on analytical performance characteristics: Linearity, Precision, Accuracy, Ruggedness, Low Limit of Detection (LOD), and Low Limit of Quantitation (LOQ) [4-8]. The validated assay methods allow for the testing of spironolactone in stability studies, using stress-factors such as acid, base, temperature, light, oxidizing agents, and susceptibility across a range of pH values, as well as dissolution testing.

**The aim** of this study was to develop a simple, sensitive, accurate, precise, and low-cost UV-spectrophotometric assay method for estimating spironolactone (S) in a combined powder that also contains potassium orotate (OK), potassium and magnesium aspartate (AsMg, AsK), and auxiliary substances.

## Materials and methods

The method was validated according to International Conference on Harmonization (ICH) guidelines.

A single beam Ultraviolet-Visible spectrophotometer (Agilent 8453, USA) with 10.0 mm matched quartz cells was used. All absorbance measurements were carried out at 25±1ºC and at 238±2nm. All weights were taken on an electronic balance (Model Radwag), and the samples were sonicated using an ultrasonic bath (Sapfir).

Potassium aspartate, magnesium aspartate, and potassium orotate (Sigma-Aldrich), spironolactone (Acros Organic and European Pharmacopoeia Reference Standard), and auxiliary substances such as microcrystalline cellulose, lactose monohydrate (Himedia), and stearic acid (Chem-Lab), as well as analytical grade reagents and solvents (Chem-Lab), were used to carry out the study.

***Preparation of the test sample***

Twenty powders were mixed, weighed, and the average weight of each powder was determined. The mass of one

powder (approximately 1.991 mg) was weighed and placed into a 25-ml volumetric flask. Approximately 10 ml of methanol was added, and the mixture was ultrasonicated for a minimum of 15 seconds before being made up to the mark with methanol. The sample was filtered through filter paper; 1 ml of this solution was transferred to a 10-ml volumetric flask and made up to the mark with methanol, resulting in a concentration of 10 µg/ml. Three series of final sample solutions were analyzed by UV spectrophotometry at 238 nm and calculated according to the Formula 1:

(1)

$$C, \% = \frac{A_{pr} * m_{st} * V_{pr} * P * (100 - U)}{A_{st} * m_{pr} * V_{st} * 100} * 100\% \quad (1)$$

$A_{pr}$ – the mean value of 3 series of absorbance of sample solutions;
$A_{st}$ – the mean value of 3 series of absorbance of standard solutions;
$m_{pr}$ – mass of sample substance, g;
$m_{st}$ – mass of standard substance, g;
P – standard substance content, %;
$V_{pr}$ and $V_{st}$ – volumes of sample and standard solutions, respectively.

***Preparation of standard stock solution***

A spironolactone standard stock solution containing 100 µg/ml was prepared in a 25-ml volumetric flask by dissolving 2.5 mg of spironolactone reference standard in methanol. The solution was sonicated for a minimum of 15 seconds and then made up to the mark with methanol. Three series of standard solutions were analyzed by UV spectrophotometry at 238 nm and calculated using Formula 1.

***Preparation of placebo solution***

The auxiliary ingredients were weighed in quantities necessary to prepare 20 powders, mixed, and homogenized in a mortar. About 1.528 mg was weighed and placed into a 25-ml volumetric flask. About 10 ml of methanol was added, and the mixture was ultrasonicated for a minimum of 15 seconds before being made up to the mark with methanol. The sample was filtered through filter paper; 1 ml of this obtained placebo solution was transferred to a 10-ml volumetric flask and made up to the mark with methanol. It was then analyzed by UV spectrophotometry.

***Validation method***

***Linearity***

Standard solutions at five different concentrations were prepared and analyzed for linearity studies to determine the linearity within the concentration range by calculating linear regression equations and regression coefficient values (Pearson, $R^2$). Linearity test solutions were prepared at levels from 50 to 300% of assay analyte concentration (5, 10, 15, 20, and 30 µg/ml). Each solution was prepared in triplicate.

*Preparation of standard calibration curves:* Aliquots of 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 3 ml from the spironolactone standard stock solution were placed into 10-ml volumetric flasks and diluted to 10ml with methanol to obtain final concentrations of spironolactone at 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 30 µg/ml, respectively. The obtained standard solutions were analyzed at 238 nm using a spectrophotometer, with methanol as a blank. The graphs were plotted with the concentration of standard spironolactone against the response (absorbance).

***Limits of detection and limits of quantification***

The limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected, while the limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be quantitated. Both LOD and LOQ were experimentally verified and calculated using the following equation: LOD = 3.3 (SD/Slope) and LOQ = 10 (SD/Slope).

***Precision***

The precision of the method was evaluated through repeatability and intermediate precision, according to intra-day and inter-day precision, and reported as percent relative standard deviation (%RSD ≤ 2). The repeatability precision was analyzed by performing six spironolactone test sample preparations made on the same day (intra-day). Intermediate precision was evaluated by 2 analysts performing the same procedure on a different day (inter-day) under the same experimental conditions.

***Accuracy***

The accuracy of the assay method was evaluated through a recovery study by adding a known amount of spironolactone standard to a pre-analyzed test sample solution at 3 different concentrations: 80%, 100%, and 120%. Various concentrations of standard spironolactone solutions of 6, 10, and 14 µg/ml were added to a fixed concentration of test sample solution (10 µg/ml) in a 1:1 ratio.

***Robustness***

The robustness of the method was determined by analyzing the sample solution (10 µg/ml) at two different wavelengths (± 4 nm) and by a single analyst performing the analysis on two different instruments, while maintaining other spectrophotometric conditions constant. The effect of these changes was studied based on the percent recovery and standard deviation of spironolactone.

The ***specificity*** of the method was assessed by evaluating the spectra of the placebo solution (containing all the

excipients of the powder except the active ingredients) to confirm the lack of interference or overlap with Spir at the analytical wavelength.

**Results**

The assay method for spironolactone in pharmaceutical forms was developed and validated according to ICH Guidelines in terms of linearity, accuracy, precision, LOD, LOQ, and robustness. Analyses were conducted using a UV-visible single-beam spectrophotometer (Model: Agilent 8453) at a wavelength of 238±2 nm, with methanol as the blank. The results are shown in Figure 2.

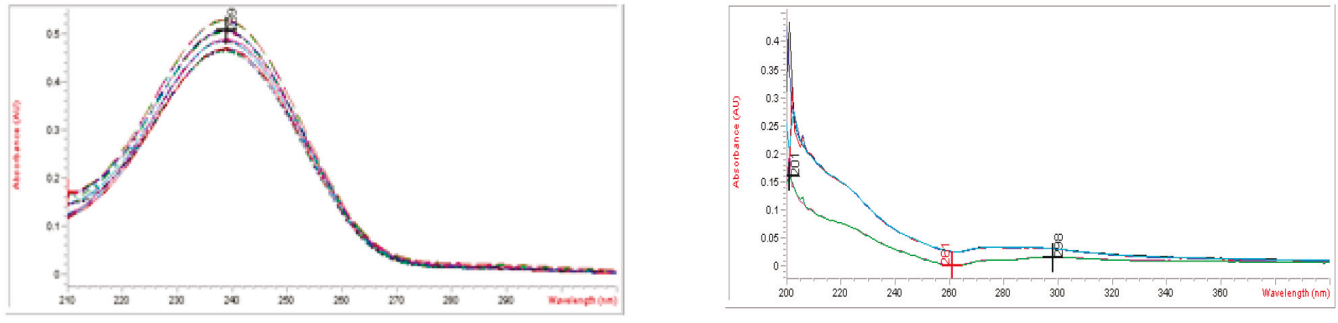


**Fig. 2** Ultraviolet absorbtion spectra of spironolactone sample (1) and placebo (2)

Linearity test solutions were prepared at 5 concentration levels, ranging from 50 to 300% of the assay analyte concentration (5, 10, 15, 20, and 30 µg/ml), and repeated 3 times (Tab.1). Linearity was established over the concentration range of 5-30 µg/ml for spironolactone. The linear regression equation was found to be: y = 0.0558x + 0.022, with an $R^2$ of 0.9994. The y-intercept (constant of regression) was 0.022, and the slope (coefficient of regression) was 0.0558. The results are represented in Figure 3.

**Table 1.** Calibration data for spironolactone spectrophotometric determination

| | Ast_1 | Ast_2 | Ast_3 | Ast_4 | Ast_5 | $Ax_{med}$ |
|---|---|---|---|---|---|---|
| 5 µg/ml | 0.279 | 0.280 | 0.279 | 0.283 | 0.279 | 0.280 |
| 10 µg/ml | 0.596 | 0.595 | 0.595 | 0.596 | 0.595 | 0.595 |
| 15 µg/ml | 0.844 | 0.844 | 0.845 | 0.845 | 0.844 | 0.845 |
| 20 µg/ml | 1.145 | 1.146 | 1.144 | 1.146 | 1.145 | 1.145 |
| 30 µg/ml | 1.673 | 1.673 | 1.688 | 1.698 | 1.698 | 1.686 |

***Note: Ast***-- Absorbance of the spironolactone standard solution at different concentrations

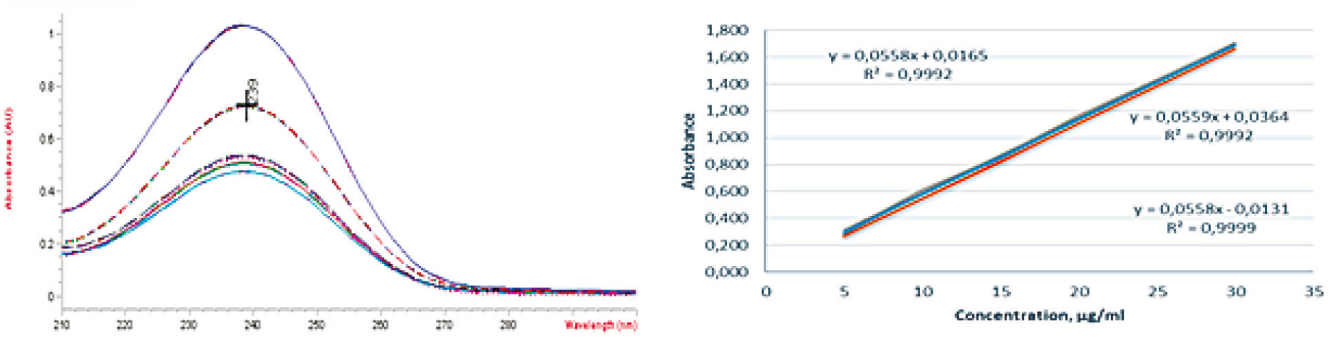


**Fig. 3** Ultraviolet spectra of standard solutions of spironolactone at different concentrations (1) and calibration curves for 3 series of determinations (2)

The determined LOD of spironolactone (0.5 µg/ml) and LOQ (1.4 µg/ml) showed the sensitivity of the method.

Precision was assessed by integrating the absorbance of a 10µg/ml test solution in six replicates on the same day and over multiple days, repeated 3 times. The precision studies showed that the % relative standard deviation was within acceptable limits (RSD < 2). The results are shown in Tables 2 and 3.

**Table 2.** Results of the repeatability precision for the assay of spironolactone

| Run | Absorbance | Amount found, % |
|---|---|---|
| 1 | 0.5261 | 100.4 |
| 2 | 0.5259 | 100.4 |
| 3 | 0.5275 | 100.7 |
| 4 | 0.5275 | 100.7 |
| 5 | 0.5273 | 100.6 |
| 6 | 0.52686 | 100.6 |
| Mean | 0.527 | 100.6 |
| RSD, % | 0.13 | 0.13 |

***Note:*** *RSD - Relative standard deviation*

**Table 3.** Results of the intermediate precision for the assay of spironolactone

| | Intra-day | | | | Inter-day | |
|---|---|---|---|---|---|---|
| Run | Absorbance of the 1st analyst | Amount found, % | Absorbance of the 2nd analyst | Amount found, % | Absorbance of the 1st analyst | Amount found, % |
| 1 | 0.5261 | 100.4 | 0.5273 | 100.6455 | 0.5259 | 100.4 |
| 2 | 0.5259 | 100.4 | 0.5269 | 100.5691 | 0.5287 | 100.8 |
| 3 | 0.5275 | 100.7 | 0.5267 | 100.531 | 0.5274 | 100.7 |
| 4 | 0.5275 | 100.7 | 0.5274 | 100.6646 | 0.5295 | 101.1 |
| 5 | 0.5273 | 100.6 | 0.527 | 100.5882 | 0.5279 | 100.8 |
| Mean | 0.527 | 100.6 | 0.5 | 100.6 | 0.5 | 100.7 |
| RSD, % | 0.15 | 0.15 | 0.05 | 0.05 | 0.25 | 0.25 |

***Note*:** *RSD - Relative standard deviation*

The accuracy of the developed method was determined by adding standard solutions (6, 10, and 14 µg/ml) to a fixed concentration of test sample solution (10 µg/ml). Accuracy was evaluated at 80%, 100%, and 120% levels of the standard solution (SD). The mean percentage recovery was calculated, and the results are shown in Table 4.

**Table 4.** Results of the accuracy for the assay of spironolactone

| Spiked Level (%) | Amount of Spir from taken sample (µg/ml) | Amount of Spir added standard (µg/ml) | Total amount of Spir (µg/ml) | Amount found of Spir from taken sample (µg/ml) ±SD | Recovery, % | RSD, % |
|---|---|---|---|---|---|---|
| 80 | 10 | 6 | 8 | *9.99*±0.08 | 99.9 | 0.82 |
| 100 | 10 | 10 | 10 | *10.07*±0.12 | 100.7 | 1.23 |
| 120 | 10 | 14 | 12 | *10.17*±0.17 | 101.7 | 1.67 |

***Note:*** RSD - Relative standard deviation, Spir - spironolactone

The robustness of the method and the influence of deliberate variations in the analytical parameters on the absorbance of Spir were examined. Parameters such as the analytical wavelength (238 ± 4 nm) and the type of instrument were modified. The test solution of 10 µg/ml Spir was applied to this parameter It was determined that these changes in the spectrophotometric determination conditions did not result in changes in the quantitative content of Spir, confirming that the developed method is robust. The results are reported in Table 5.

**Table 5.** Results of the robustness for the assay of spironolactone

| | Parameter | RSD, % | Assay, mg | Assay, % |
|---|---|---|---|---|
| Wavelength | 234 nm | 0.17 | 23.68 | 100.8 |
| | 238 nm | 0.15 | 24.14 | 100.6 |
| | 242 nm | 0.07 | 23.69 | 99.1 |
| Instrument | Agilent 8453 | 0.15 | 24.14 | 100.6 |
| | Shimadzu 1800 UV | 0.13 | 24.64 | 102.68 |

**Note:** RSD - Relative standard deviation, Spir - spironolactone

In the presence of common excipients such as microcrystalline cellulose, lactose monohydrate, and stearic acid, no interferences were observed (Figure 2). This confirms the specificity of the developed method.

## Discussions

In this study, a validated method was developed for the analysis of spironolactone from a new compound powder containing spironolactone, potassium orotate, potassium and magnesium aspartate, and excipients. This combination of active pharmaceutical substances is specific and can be successfully applied for the treatment of hypopotassemia. According to other reported studies, different solvents (such as ethanol, methanol, acetonitrile) and wavelengths have been used to analyze spironolactone from other medicines by UV-Vis spectrophotometry. Based on the performed analyses, we selected the most satisfactory conditions to validate the method in compliance with ICH guidelines for specificity, linearity, precision, accuracy, and robustness. This method can be applied for routine quality control, quantitative analysis, and stress stability testing. The UV-Vis spectrophotometric method for the analysis of spironolactone from the compound powder was developed for the first time and demonstrated good accuracy, precision, LOD, LOQ, robustness, and specificity. The results of the analysis were statistically validated using SPPS, and the RSD (%) values for all measurements were less than 2.

## Conclusion

The proposed UV-Vis spectrophotometric method was found to be simple, rapid, precise, and low-cost. Validated according to ICH guidelines, the method demonstrated ex-

cellent linearity, accuracy, precision, LOD, LOQ, robustness, and specificity. This new analytical method was developed for the routine simultaneous determination of spironolactone in the presence of OK, AsK, and AsMg in the newly developed pharmaceutical form – combined powder.

**Competing interests**

The author declares that there is no conflict of interest in the manuscript.

**Acknowledgements and funding**

The research was conducted at the Scientific Center for Drug Research within *Nicolae Testemiţanu* State University of Medicine and Pharmacy. The study was initiated by the author.

**Authors' contributions**

EM conducted the literature review, wrote the manuscript, revised the final text, and approved the final version of the manuscript.

**Ethics approval**

Not needed for this study

**References**

1. Ghicavîi V, Bacinschi N, Guşuilă G. Farmacologie [Pharmacology]. 3rd ed. Chişinău: Print Caro; 2019. 1000 p. Romanian.
2. ChemSpider. Spironolactone: chemical structure [Internet]. London: Royal Society of Chemistry; 2024- [cited 2024 Apr 5]. Available from: http://www.chemspider.com/Chemical-Structure.5628.html
3. ChemSpider. Aldosterone: chemical structure [Internet]. London: Royal Society of Chemistry; 2024- [cited 2024 Apr 5]. Available from: http://www.chemspider.com/Chemical-Structure.5633.html
4. International Conference on Harmonization Expert Working Group. Validation of analytical procedures: text and methodology Q2(R1): ICH Harmonised Tripartite Guideline [Internet]. Geneva: ICH; 2005 [cited 2024 Apr 5]. Available from: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf
5. Uncu L, Donici E, Valica V, Vîslouh O, Gonciar V, Parii S. Development and validation of an assay method for ciprofloxacin hydrochloride determination in combination ear drops. Chem J Mold. 2019;14(2):56-61. https://doi.org/10.19261/cjm.2019.607.
6. Prajapati P, Chandarana C, Modi V, Basuri T. Quantification of Spironolactone by first and second order UV Derivative Spectrophotometry in bulk and tablet dosage form. Int J Pharm Chem Anal. 2016:3(3):168-173. doi: 10.5958/2394-2797.2016.00024.1.
7. Chaudhary A, Vadalia KR, Thummer P. Development and validation of ratio derivative spectrophotometric for simultaneous estimation of metolazone and spironolactone in pharmaceutical dosage form. Int J Pharm Sci Res. 2012;3(10):3999-4003.
8. US Food and Drug Administration. Guidance for industry: Bioanalytical method validation, 2001 [Internet]. Washington: FDA; 2001 [cited 2024 Apr 5]. Available from: http://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf