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Experimental Biomedical Research **Original article**

Development and validation of UV/VIS spectroscopy method for determination of atezolizumab in pharmaceutical products

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ABSTRACT

Aim: The aim of this study was to develop and validate a simple, fast, and reliable UV visible methodology for the determination of atezolizumab in pharmaceutical products.

Methods: The maximum wavelength of atezolizumab was determined using a UV/Vis spectrum and the calibration curve has been established. Validation studies were carried out to determine the reliability of the spectrophotometer method used in quantification of pharmaceutical products.

Results: According to the experimental data, the developed method was linear in a range varying from 0.10 to 1.50 $mg.mL^{-1}$ determined by 6 individuals calibrations points. The r^2 value was 0.9995 indicating a 99.95% correlation in linearity and precision. The robustness showed good and similar values and the limit of detection and limit of quantification were 0.005 $mg.mL^{-1}$ and 0.018 $mg.mL^{-1}$, respectively.

Conclusion: The data corroborates the reliability as applicability of the developed UV/Vis spectroscopy method for quantitatively determining the amount of atezolizumab in pharmaceutical products.

Key words Atezolizumab, monoclonal antibody, UV/Vis spectroscopy, validation, pharmaceutical products.

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Introduction

Atezolizumab is a monoclonal antibody and has been approved by FDA for non-small cell lung cancer, metastatic urothelial carcinoma and triple negative breast cancer [1]. Preclinical studies showed that atezolizumab has a dose-dependent pharmacokinetic profile in intervals of dose from 0.5 to 5.0 $mg.kg^{-1}$. Nonetheless in higher dose (5–20 $mg.kg^{-1}$) a linear behavior is observed [2]. The therapeutic protocol recommends a dose of 1200 mg (approximately 15 $mg.kg^{-1}$) given over 1-h by intravenous infusion every 21 d. Although the therapeutic used approved, atezolizumab shows a higher number of adverse reactions, which includes: fatigue, cough, decreased appetite, dyspnea, musculoskeletal pain, constipation, nausea, pyrexia, diarrhea, arthralgia, rash, anemia, lymphocytopenia, hypoalbuminemia, hyponatremia, elevated alkaline phosphatase concentrations, elevated ALT/AST concentrations, hypophosphatemia and hypomagnesemia [3].

In recent years, monoclonal antibodies has been used in several clinical approaches including cancer therapy [4-7], chronic diseases [8-11] and many other applications [12-14] and

researchers round the world can design monoclonal antibodies to a specific target, such as overexpressed tumor proteins. One of the main issues regarding monoclonal antibodies is the precise determination. Although, there are several techniques used for monoclonal antibodies quantification most of them are expensive or take a long time to be processed [15]. Thus, fast, reliable and cheap techniques as UV spectrophotometry [16] to quantify precisely monoclonal antibodies are required and important technological approach, especially for pharmaceutical products.

Regarding the atezolizumab, there is no UV spectrophotometry methodology published for the quantification of atezolizumab in pharmaceutical preparations. The methodologies found in literature to quantify this monoclonal antibody are: reverse – phase separation high performance liquid chromatography (RP-HPLC) [17], capillary zone electrophoresis (CZE) [18], two-dimensional liquid chromatography-tandem mass spectrometry (2D-LC-MS/MS) [19] and ion mobility mass spectrometry (IM-MS) [20].

The aim of our study was to develop a rapid, accurate and reliable UV/Vis method, with a simple, repeatable and inexpensive technique for the quantitative determination of atezolizumab in pharmaceutical products.

Materials and methods

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-220/S/361) within the scope of PhD thesis of Meliha Ekinci.

Materials: Atezolizumab (Tecentriq) was obtained from La Roche/Genentech. The saline solution (0.9% sodium chloride solution) was obtained from Intermountain Life Sciences, LLC. UV quartz spectra cuvette was obtained from Hellma GmbH, Germany.

UV/Vis Spectrum: The maximum wavelength was determined using a UV/Vis spectrum (Beckman Coulter DU® 730 Life Science UV/Vis Spectrofotometer) in the range of 200-400 nm from the solution of atezolizumab prepared in 6 series in 1.50 $mg.mL^{-1}$ saline medium [21].

UV/Vis Method: The calibration curve has been established. In this direction, standard solution of atezolizumab at concentration of 0.10, 0.25, 0.50, 0.75, 1.00 and 1.50 $mg.mL^{-1}$ were prepared and measured using a Beckman Coulter DU® 730 Life Science UV/Vis Spectrofotometer using the maximum wavelength (λ_{max}), previously determined.

Validation: Validation studies were carried out to determine the reliability of the spectrophotometer method used in quantification of pharmaceutical products. Thus, linearity, accuracy, precision (repeatability), durability (stability), specificity (selectivity), consistency, robustness, LOD and LOQ (sensitivity) were determined, as preconized FDA [22,23].

Validation Parameters

Linearity

The calibration curve has been determined according to Jaccoulet *et al.* [21]. In this direction atezolizumab at concentrations of 0.10, 0.25, 0.50, 0.75, 1.00 and 1.50 $mg.mL^{-1}$ (n = 6) were prepared, and the absorbance of each sample were determined by UV/Vis (Beckman Coulter DU® 730 Life Science UV/Vis Spectrofotometer). Finally, the r^2 were calculated to establish the correlation parameter.

Accuracy

To show the accuracy, the concentration used and the calculated one for 3 points (0.10, 0.75 and 1.50 $mg.mL^{-1}$) were selected. The accuracy value was determined as percent (%) both intraday and inter-days (**Eq. 1**).

(Eq. 1): (Practically calculated average concentration value / theoretically calculated concentration value) × 100

Precision (Repeatability)

For repeatability, at the concentrations of 0.10, 0.75 and 1.50 mg.mL^{-1} atezolizumab solution was prepared, and UV/Vis absorbance was measured 5 times consecutively from the same sample both intraday and inter-days. As a result of these measurements, the area values of atezolizumab were compared and means, standard deviations and relative standard deviations were calculated.

Durability (Stability)

Six parallel standard solutions of a 0.75 mg.mL^{-1} concentration of atezolizumab were prepared and their absorbance at UV/Vis was measured in the same order at the start time and 24-h after waiting at 4°C.

Originality (Selectivity)

To determine whether the analytical method used for the determination of the specificity of the substance belongs only to atezolizumab, the absorbance in UV/Vis of the solution containing atezolizumab and no atezolizumab was measured and the obtained spectrums were examined.

Consistency

The consistency of the method was tested by comparing the results obtained from the absorbance measured in UV/Vis of a 0.75 mg.mL^{-1} medium concentration of atezolizumab, prepared by two different individuals.

Robustness

The robustness of the method was tested by comparing the results obtained by changing and measuring conditions and optimized conditions of atezolizumab at a medium concentration of 0.75 mg.mL^{-1} in UV/Vis.

Sensitivity

Whether the developed method is sensitive enough for atezolizumab was evaluated by calculating LOD and LOQ.

Statistical analysis

Statistical analysis and variance analyze (Univariate Variance Analyze) of all the obtained results were done using SPSS software version 25. The statistical significance level was accepted as $P < 0.05$ for all analyses performed. Results were obtained in triplicate and presented as the mean ± SD.

Results and Discussion

UV/Vis Spectrum

The maximum absorbance for atezolizumab has been determined and the value found was 280 nm (Figure 1). This is the first time that this value has been described.

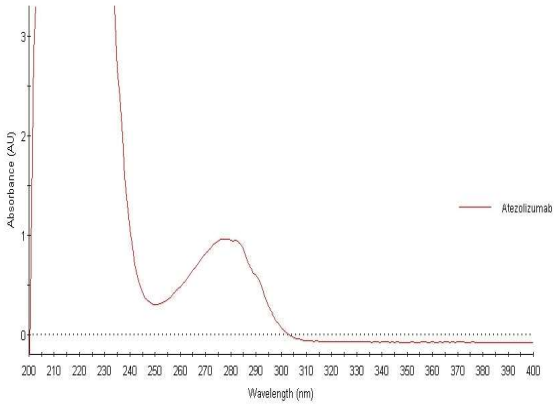

Figure 1. UV/Vis spectrum of atezolizumab.

Considering the protein structure from the monoclonal antibody, which usually have a λ_{max} varying from 245-300 nm [21,24-27], our data (280 nm) is quite consistent. Also, we believe that this data will contribute to the literature and help researchers around the world to easily determine atezolizumab in the nanosystems.

Validation

Validation studies were carried out to determine the reliability of the spectrophotometer method used in quantification. Linearity, accuracy, precision (repeatability), durability (stability), specificity (selectivity), consistency, robustness and sensitivity were determined as validation parameters according to the criteria recommended by the FDA [22,23,28,29].

Validation parameters

Linearity

For the linearity parameter, 6 different concentrations of atezolizumab solution were prepared by diluting the stock solution and their absorbance in UV/Vis was measured. The calibration curve drawn with the help of the obtained absorbance values are shown in **Figure 2**. The line equation was y = 1.4542x - 0.0397 and the r^2 value 0.9995. This value indicates that the line shows 99.95% linearity [22,23].

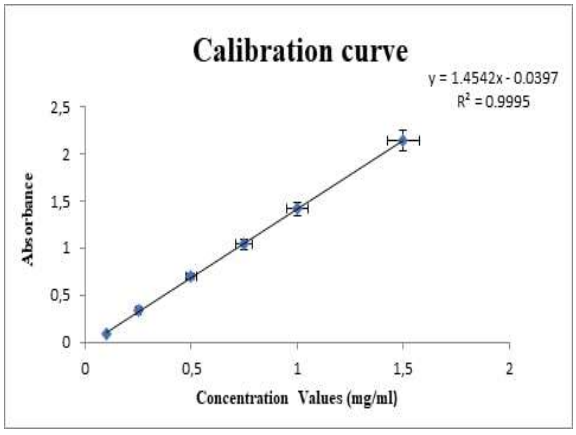

Figure 2. Calibration curve of atezolizumab.

Accuracy

To demonstrate the accuracy of the analytical method used, the absorbance values measured by UV/Vis analysis both intraday and inter-days were measured based on the amount of atezolizumab prepared at known concentrations (0.10, 0.75 and 1.50 $mg.mL^{-1}$) and its proximity to the actual value was determined as a percentage (%) accuracy value. The data obtained are shown in Table 1 and Table 2.

Table 1. Intraday accuracy values (%) calculated for atezolizumab.

	Concentration Values ($mg.mL^{-1}$)		
	0.10 $mg.mL^{-1}$	**0.75 $mg.mL^{-1}$**	**1.50 $mg.mL^{-1}$**
Average	0.098	0.752	1.477
SD	0.002	0.004	0.005
RSD (%)	1.793	0.543	0.350
Accuracy (%)	97.67	100.22	98.44

Table 2. Inter-days accuracy values (%) calculated for atezolizumab.

	Concentration Values ($mg.mL^{-1}$)		
	0.10 $mg.mL^{-1}$	**0.75 $mg.mL^{-1}$**	**1.50 $mg.mL^{-1}$**
Average	0.098	0.752	1.48
SD	0.001	0.004	0.006
RSD (%)	0.830	0.543	0.427
Accuracy (%)	98.33	100.22	98.67

As a result of the six parallel injections at the same concentration, the intraday and inter-days (%) accuracy values are 97.67, 100.22, 98.44% and 98.33, 100.22, 98.67%, respectively. In the statistical evaluation, no significant difference was observed between the intraday and inter-days accuracy values calculated from six injections for each concentration, as well as between low, medium, and high concentrations ($P > 0.05$). The expected accuracy value should be greater than 80% in the validation studies carried out by the FDA and the relative error should not be higher than 2. So, the accuracy study met this criterion [22,23].

Precision (Repeatability)

For repeatability, atezolizumab solution was prepared at 0.10, 0.75 and 1.50 $mg.mL^{-1}$ concentrations and UV/Vis measurement were performed 5 times consecutively from the same sample both intraday and inter-days. As a result of these measurements, the average, standard

deviation, and relative standard deviation values calculated by comparing the area values of atezolizumab are given in Table 3 and Table 4.

The percentage (%) of RSD values were calculated (1.333, 0, 0.371% and 0.557, 0.560, 0.371%) intraday and inter-days, respectively, and were found within the desired limits (not higher than 2) for each concentration [22,23].

Table 3. Intraday repeatability values (%) calculated for atezolizumab.

	Concentration Values (mg.mL^{-1})		
	0.10 mg.mL^{-1}	**0.75 mg.mL^{-1}**	**1.50 mg.mL^{-1}**
Average	0.098	0.75	1.476
SD	0.001	0	0.005
RSD (%)	1.333	0	0.371
Accuracy (%)	97.80	100.00	98.40

Table 4. Inter-days repeatability values (%) calculated for atezolizumab.

	Concentration Values (mg.mL^{-1})		
	0.10 mg.mL^{-1}	**0.75 mg.mL^{-1}**	**1.50 mg.mL^{-1}**
Average	0.098	0.748	1.476
SD	0.001	0.004	0.005
RSD (%)	0.557	0.560	0.371
Accuracy (%)	98.40	99.73	98.40

Durability (Stability)

6 parallel standard solutions of atezolizumab at 0.75 mg.mL^{-1} concentration were prepared and their absorbance in UV/Vis was measured at the start time (0-h) and 24-h in the same order. The results obtained were given at Table 5.

Table 5. Stability test results.

	Time (h)	
	Initial (0-h)	**24-h**
Average	0.75 mg.mL^{-1}	0.748 mg.mL^{-1}
SD	0	0.004
RSD (%)	0	0.546
Accuracy (%)	100.00	99.78

The RSD values were found to be 0% and 0.546%, respectively, according to the results obtained from the injections made at the time of start (0-h) and 24-h. It has been suggested by the FDA that % RSD values should be less than 20% for low concentrations and 15% for medium and high concentrations. Accordingly, % RSD values were found within the desired limits [22,23].

Originality (Selectivity)

To determine whether the analytical method used for the determination of the originality of the substance belongs only to atezolizumab, the absorbance of the saline solution containing atezolizumab and no atezolizumab in UV/Vis was measured and the spectra obtained are given in Figure 1 and Figure 3.

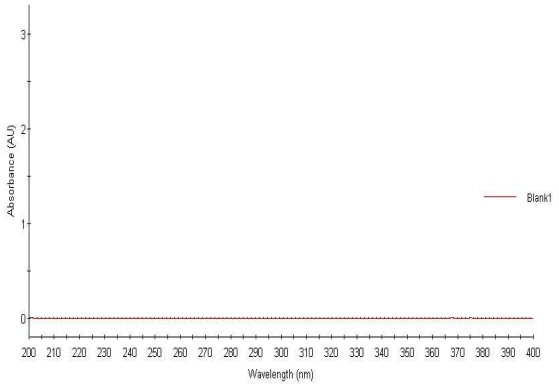

Figure 3. Spectrum of solution without atezolizumab.

The parameter of the specificity showed a maximum absorbance peak at 280 nm (Figure 1) corresponding to atezolizumab. Nonetheless, the sample free of atezolizumab showed no peak (Figure 3), indicating the selectivity of the method as preconized by FDA [22,23].

Consistency

The consistency of the method was evaluated by comparing the average of the results obtained from the absorbance measured in UV/Vis of atezolizumab at the medium concentration of 0.75 mg.mL^{-1} prepared by two different people and there was no statistically

significant difference between the averages (P > 0.05). Thus, consistency parameter meets the FDA criteria [22,23].

Robustness

The robustness of the method was tested by comparing the results obtained by changing the conditions and repeating the measurement (from the medium concentration of 0.75 $mg.mL^{-1}$) using UV/Vis. The results obtained from optimized conditions were evaluated and no statistically significant difference between the averages (P > 0.05) were found. The data corroborates the high robustness of the methodology [22,23].

Sensitivity

Whether the developed method is sensitive enough for atezolizumab was evaluated by calculating the limit of detection (LOD) and limit of quantification (LOQ). The results are given in Table 6. The LOD value of the atezolizumab was found to be 0.005 $mg.mL^{-1}$ and a LOQ value of the atezolizumab was found to be 0.018 mg/ml using the lowest atezolizumab concentration possible (Table 6).

Table 6. Sensitivity test results.

Parameter	Concentration Value ($mg.mL^{-1}$)
Linearity Range	0.10-1.50 $mg.mL^{-1}$
LOD	0.005 $mg.mL^{-1}$
LOQ	0.018 $mg.mL^{-1}$

The sensitivity parameter, which has the ability to distinguish small deviations in the concentration of active substance or to detect low concentrations of the active substance, was found to be sensitive enough for atezolizumab.

Conclusion

As a result of all the parameters obtained, the UV/Vis method was found to be linear, highly accurate, reproducible, stable, unique, consistent, high robust and sensitive according to the criteria specified by the FDA. These data show that the developed UV/Vis spectroscopy method is a suitable method for quantitatively determining the amount of atezolizumab in pharmaceutical products and also in commercial samples, various dosage forms and rat-mice blood/plasma and human specimens.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *Ethics committee decision was not taken as it was a spectroscopy method.*

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