ORIGINAL ARTICLE

A C G publications

J. Chem. Metrol. 18:1 (2024) 10-18

journal of chemical metrology

Development of an RP-HPLC method to evaluate the basic characteristics of talazoparib-loaded PLGA nanoparticles

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(Received October 27, 2023; Revised January 16, 2024; Accepted January 19, 2024)

Abstract: The use of poly (ADP-ribose) polymerase (PARP) inhibitors for cancer treatment has been reported previously. Talazoparib is a PARP inhibitor, and its solubility problems encouraged us to prepare talazoparib-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles for use in brain cancer models. To determine the encapsulation efficiency and release profile, a reversed-phase high-pressure liquid chromatography (RP-HPLC) method was developed and validated. A Shiseido 5 µm C18 100 Å column (250 × 4.6 mm) was used with a flow rate of 1.0 mL/min. Isocratic elution was performed using an acetonitrile:phosphate buffer (100 mm, pH 6.25) (35:65 v/v) mixture. The injection volume was 5 µL and UV detection was performed at 227 nm. The method was linear within the range from 0.1 to 12.5 µg/mL. The encapsulation efficiency and release profile of the prepared formulation were analyzed using the developed RP-HPLC method, and it was found that the encapsulation efficiency was 65.17% ± 0.50 and the release of the talazoparib was around 40% within 5 h and remained stable for 25 h. The RP-HPLC method developed in the present study can be adapted for further applications to determine talazoparib in its commercial formulations and proposed encapsulated drug delivery systems.

Keywords: Talazoparib; RP-HPLC; nanoparticles; drug delivery; method validation.

1. Introduction

According to World Health Organization (WHO) figures, cancer is the first or second major cause of death before the age of 70 in 112 of 183 nations, and it ranks third or fourth in another 23 [1]. Among cancers of many types, the treatments developed are very valuable due to factors such as brain tumors, the scarcity of drugs that can be used in brain tumors, the complexity of brain functioning, and the blood–brain barrier.

Olaparib, rucaparib, talazoparib, and veliparib are examples of poly (ADP-ribose) polymerase (PARP) inhibitors, which are newly developed molecules. The PARP family comprises nuclear enzymes that play a role in recognizing and repairing DNA single-strand breaks. Talazoparib, on the other hand, is a chemotherapeutic, orally administered drug that inhibits PARP in solid tumors.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology January-June 2024 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.2310.2937
Available online: February 24, 2024

The IUPAC name of talazoparib is (11S,12R)-7-fluoro-11-(4-fluorophenyl)-12-(2-methyl-1,2,4-triazol-3-yl)-2,3,10-triazatricyclo[7.3.1.05,13]trideca-1,5(13),6,8-tetraen-4-one and its molecular weight is 380.4 g/mol.

Nanoparticle-mediated targeted delivery of drugs is a key option to reduce the dosage of drugs, besides having the capability of controlled release. These systems also decrease toxicity, enhance shelf life, and boost specificity and bioavailability. Tumor vessels have abnormal physiological structures, and this makes tumor tissues hard to reach to be affected by drugs. In addition, medications also face challenges in crossing the blood–brain barrier during the treatment of brain cancers. However, nanoparticles have the capacity to achieve this as well. Nanoparticles overcome all these significant impediments and enhance targeting solid tumors with permeability and impact. The development of nanocarrier systems for targeting brain tumors is, therefore, critical [2].

Talazoparib is soluble in dimethylformamide and almost insoluble in water [3]. Although it is thought to have low solubility and medium permeability, there is no clear information in the literature about which class it is in the Biopharmaceutical Classification System [4]. However, it is proposed to be used together with nanoparticle systems that are able to pass through the blood–brain barrier and stop the decline in bioavailability due to solubility problems [5].

As it is known, there are a wide variety of drug delivery systems. The main ones are polymers, lipidic nanoparticles, metallic nanoparticles, and micelles. Polymeric nanoparticles are one of the most commonly used types, and poly(lactic-co-glycolic acid) (PLGA), a synthetic polymer, is often used to prepare nanoformulations [6]. Embedding PLGA into the nanoparticle can prevent the solubility problem and ensure that it stays in circulation for a longer time and reaches the effect site, thus increasing its bioavailability.

During the development of these systems, the crucial point is to ensure the characterization parameters of the formulation. Thus, the behavior of the nanoparticle-mediated targeted delivery of talazoparib could be considered when a real application in cancer therapy is administered.

In the present study, a polyacrylic acid (PAA)–PLGA formulation of talazoparib was developed based on a modified methodology used in the literature, and an RP-HPLC technique to ascertain talazoparib was developed to evaluate its characteristics, including encapsulation efficiency and drug release profile [7].

There are very few HPLC methods for talazoparib in the literature, but there are methods that achieve analysis of talazoparib in plasma samples [8, 9]. Hidau et al. developed a validation method for talazoparib. They used RP-HPLC. Their matrix was rat plasma (biological sample). The chromatographic conditions of the method were as follows: UV 227 nm, total run 10 min, flow rate 1 mL/min, extraction solution MeOH:ACN (65:35), linearity range 0.1-2.0 µg/mL, and recovery was higher than 85% [8]. Liquid chromatography-mass spectrometry (LC-MS) was used to determine the presence of talazoparib in pharmaceutical dosage forms in recent research by Pakalapati et al. [10]. The published analytical methods to determine talazoparib were specific to biological samples, or they employed LC-MS to determine the active ingredient. There was no RP-HPLC method reported previously for analyses of the formulations. Considering the potential contributions of talazoparib to tumor therapy, the aim was to create a technique to measure talazoparib in PLGA formulations. The specificity, linearity, range, accuracy, precision, and robustness of the novel method were assessed using the ICH recommendations [11]. The proposed methodology was effectively used to measure talazoparib in the PAA–PLGA formulation to calculate the encapsulation efficiency and release profile of the drug. The analytical method developed in the present study is the first reported to analyze talazoparib in a nanoencapsulated formulation using HPLC, and the method can be transferred to further applications in which talazoparib is used as the active pharmaceutical ingredient.

2. Experimental

2.1. Chemicals and Reagents

Tetradecafluorohexane (99.0%) (TDFH), polyvinyl alcohol (PVA), PAA, dichloromethane (99.8%) (DCM), and PLGA 503H were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grades of methanol (99.9%) (MeOH) and ACN (99.9%) were purchased from Merck (Darmstadt, Germany). Talazoparib (98.0%) was provided by Cayman Chemicals. All the solutions were prepared using Milli-Q water.

2.2. HPLC Conditions

The HPLC of the Agilent Technologies 1200 Series system consisted of a G1311A quat pump, a G113A degasser, an automatic sampler, and a DAD detector. The detector was set at 227 nm, and peak areas were integrated automatically by a computer equipped with Agilent ChemStation.
The phosphate pH 6.25 buffer used for HPLC analysis was prepared as follows: 15.6 mg of sodium phosphate monobasic dihydrate ($NaH_2PO_4.2H_2O$) was dissolved in pure water. The volume was around 800 mL. With the addition of 0.1 N sodium hydroxide (NaOH), the pH of the solution was brought to 6.25, and the volume of the final solution was made up to 1 L with clean water.
Separation was carried out in a Shiseido 5 µm C18 100 Å column (250 × 4.6 mm). Using isocratic elution and a 35:65 v/v combination of ACN and buffer, the flow rate was 1.0 mL/min. UV detection at 227 nm was carried out using an injection volume of 5 µL.

2.3. Preparation of the Calibration Curves

Talazoparib standard stock solution was created in a 60:40 v/v MeOH:water combination at a concentration of 100 µg/mL. The calibration curves of talazoparib were constructed under optimum conditions, and the linearity of the method was determined by performing injections at seven different concentration levels (0.1, 0.25, 0.5, 1.0, 5.0, 10.0, and 12.5 µg/mL). The peak areas of talazoparib were plotted against the corresponding concentrations of talazoparib to obtain calibration graphs. The standard deviations of the slope and intercepts were calculated beside the average regression coefficient.

2.4. Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) for the developed method were measured by the use of signal-to-noise ratio (S/N: 10 for LOQ and 3 for LOD). These parameters are crucial for determining the analytical method's sensitivity and performance bounds, as well as the smallest amount of the target analytes at which accurate detection and quantification are possible.

2.5. Precision and Accuracy

The RP-HPLC method's accuracy and precision were carefully assessed to verify the reliability and reproducibility of the analytical measurements. Talazoparib samples spiked into extracted matrices of PAA–PLGA nanoparticles at three different concentrations (0.1, 5.0, and 10.0 µg/mL) (n=3), including the LOQ value, were determined using the developed RP-HPLC method. Precision and accuracy were assessed using the relative standard deviation (RSD) of the measurements and comparing the observed amounts to the spiked samples' known concentrations.

2.5. Preparation of the Formulation

PAA–PLGA nanoparticles were prepared by the solvent evaporation/extraction method. Talazoparib-entrapped methylcellulose nanoparticles and TDFH (a kind of perfluorocarbon) were encapsulated by the PLGA. The formulation was coated with PAA [7].

2.6. Encapsulation Efficiency of the PAA–PLGA Formulation

The encapsulation efficiency of the PAA–PLGA formulation was determined using an indirect method [12]. First, 2 mL of the prepared formulation was centrifuged for 45 min at 13,000 rpm. Consequently, 500 µL of the supernatant was taken and diluted with 500 µL of MeOH:water (60:40 v/v)

and determined by HPLC. The remaining amount of talazoparib in the supernatant enabled us to determine the encapsulation efficiency of the formulation.

2.7. Release Studies of the PAA–PLGA formulation

Release studies were performed for the PAA–PLGA formulation since it was designed to provide controlled release of the active substance. The formulation, which was planned to be administered intravenously, was studied in a pH 7.4 release environment to mimic the blood environment.

Firstly, the sink condition was determined with a solubility experiment. Thus, the amount of formulation we must use in the drug release was calculated. The drug release experiment was carried out with the dialysis membrane method (14,000 molecular weight cut-off) [13]. First, 1 mL of PAA–PLGA was added between the membranes, and it was replaced with a 15 mL Falcon tube. Then 10 mL of pH 7.4 phosphate buffer saline (PBS) buffer was added between the Falcon tube and the dialysis membrane. In a shaker machine, the temperature was fixed at 37 °C. Samples were collected at 0.25, 0.50, 1, 2, 4, 8, 12, and 24 h. The collected samples were directly vialed and analyzed.

2.8. Estimation of Uncertainty Budget

Uncertainty is referred to as "a parameter, associated with the result of a measurement, which characterizes the dispersion of the values that could reasonably be attributed to the measurand." The concentration of an analyte is frequently referred to as the measurand in chemical analysis. Uncertainty refers to a lack of confidence in an analytical outcome. Consequently, uncertainty provides understanding of the significance of the outcome by displaying the range of values that the analyst feels represent the "true concentration" of the analyte.

The uncertainty assessment for the method was performed as per the EURACHEM/CITAC guide and the corresponding literature [14]. Uncertainty was calculated for $u_{standard}$, $u_{calibration}$, $u_{recovery}$, and $u_{repeatability}$. u_{combined} and $U_{expanded}$ values were obtained using the other parameters.

The calculations are shown below.

u_{combined} is calculated from the other parameters' square root of the sum of squares.

$$u_{Combined} = \sqrt{(u_{Standard})^2 + \left(u_{Linearity}\right)^2 + (u_{Recovery})^2 + (u_{Repeatability})^2} \quad (1)$$

u_{Standard} of the analyte was calculated regarding the standard talazoparib purity (Equation 2).

$$u_{Standard} = \frac{100 - \%Purity}{\sqrt{3}} \quad (2)$$

$U_{\text{Linearity}}$ was determined using equation 3 for each analyte based on the slope value for the calibration curve and the standard error of the slope.

$$u_{Linearity} = \frac{(Standard\ Error\ of\ Slope * 100)}{Slope} \quad (3)$$

The mean relative standard deviation (RSD) associated with the recovery studies was considered $u_{recovery}$, while that of the repeatability studies was considered $u_{epeatability}$ for the analyte. Expanded uncertainty ($u_{expanded}$) at a 95% confidence interval was calculated by multiplying combined uncertainty with the coverage factor (k = 2).

3. Results and discussion

3.1 Method Development and Optimization

RP-HPLC method for talazoparib in nanoparticles

Talazoparib is a chemical compound with amine groups and aromatic rings. Its Log P value was reported as 2.11 based on DrugBank (www.drugbank.ca) data. Its relatively nonpolar features allow us to use a C18 column to perform the experiments. In the reported HPLC study, MeOH–ACN (65:35) in deionized water was used as the mobile phase for separation in a C18 column [8]. In our method development step, mobile phase pH (pH 5.00 and 6.25 selected based on initial experiments), different mobile phase ratios, and wavelengths were evaluated to find the optimum conditions. The optimum conditions were found while using an isocratic elution where the mobile phase was ACN:phosphate buffer [0.1 M, pH 6.25] (35:65 v/v). The flow rate was 1 mL/min and the injection volume was 5 µL. Based on the studies in the literature, analyses at four different wavelengths (227, 254, 309, and 365 nm) were utilized [12-13]. Finally, the optimum wavelength for analysis was selected as 227 nm, where we achieved a better response when the method was still selective for talazoparib in our experimental conditions. The system suitability parameters under optimum conditions were retention time (min) 6.9; capacity factor (k') 1.61; efficiency (N) 11638; peak symmetry 0.77. The chromatograms for standard talazoparib and blank nanoparticles are given in Figure S1 (see supporting information).

3.2. Method Validation

3.2.1. Selectivity

Selectivity is the ability to specifically separate substances to be analyzed in the medium without any interference (matrix, formulation, etc.) while using the applied method. The active substance, the solvent of the active substance, and the substances to be used in the formulation were analyzed sequentially and compared with each other using overlapped chromatograms. According to the data obtained, there was no conflict in the retention time of any substance (PLGA, Tween 80, methylcellulose, and PVA) coming from matrix components (Figure 1 and Figure S1). The method was selective in our experimental conditions.

3.2.2. Linearity

An analytical process is considered to be linear if it generates test results that are precisely correlated to the concentration of the analyte in the sample within a certain range. The method was linear between 0.1 and 12.5 µg/mL. The calibration equation was $y = 12.53\ (\pm 0.09)\ x + 0.05\ (\pm 0.06)$, where y is the peak area and x is the talazoparib concentration expressed in µg/mL. The regression coefficient was 0.9990.

3.2.3. LOD and LOQ

The LOD serves the lowest concentration at which the analyte signal is distinguishable from the background noise. LOQ, on the other hand, is the lowest level concentration at which the analyte can be reliably quantified with acceptable accuracy, precision, and linearity. The S/N ratio method involves comparing the signal (analyte response) to the noise (background variability). By establishing a threshold S/N ratio, the signal can be reliably distinguished from the background noise. This method ensures that the reported measurements are above the noise level, guaranteeing accuracy and reliability in quantification.

Based on the S/N ratios of the chromatograms, LOQ and LOD were 0.1 µg/mL and 0.03 µg/mL, respectively. The representative chromatograms to show the linearity of the method are given in Figure 1.

3.2.4. Precision and Accuracy

The obtained values for the RSD% and biases of the spiked samples are summarized in Table S1. As seen from the results, the RSD% was lower than 1.0% for three concentration levels and the mean accuracy values were between 100.36% and 100.70%. These results indicate that the developed method was satisfactory for determining talazoparib in the PAA–PLGA formulation.

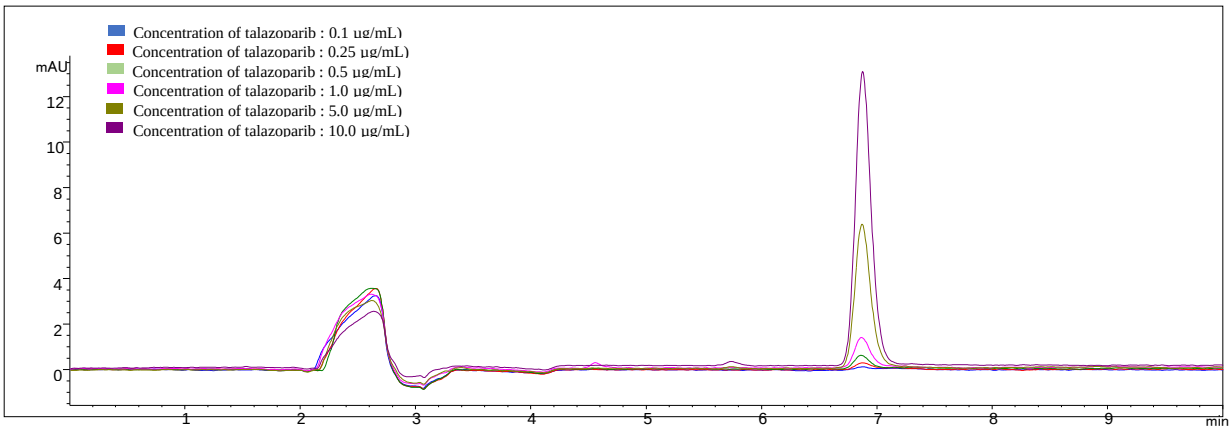

Figure 1. Chromatograms of talazoparib standards (0.1, 0.25, 0.5, 1, 5, and 10 µg/mL) under optimum conditions: Shiseido 5 µm C18 100 Å column (250 × 4.6 mm). Flow rate: 1.0 mL/min [isocratic elution with ACN:buffer (35:65) mixture], injection volume: 5 µL, UV detection: 227 nm.

3.3. Estimation of Uncertainty Budget

The significance of uncertainty assessment, as outlined in the EURACHEM/CITAC guide, lies at the core of ensuring reliable and credible measurement results. This process is not merely about acknowledging potential errors; it is a structured methodology to quantify and comprehend the inevitable doubts inherent in any measurement. By meticulously evaluating uncertainty, this guide enables a comprehensive understanding of the limitations and variability within measurement processes. It provides a standardized framework to identify and assess sources of uncertainty, integrating statistical tools and calibration data to generate robust uncertainty estimates.

There are several sources of uncertainty related to the EURACHEM/CITAC guide that may be categorized, including sampling, storage, instrument effects, reagent purity, assumed stoichiometry, measurement conditions, sample effects, computational effects, blank correction, operator effects, and random effects.

In order to determine the uncertainty, the EURACHEM/CITAC guide was employed with the results for the validation parameters of the study. Accordingly, $u_{Standard}$ was calculated as 0.11, $u_{Linearity}$ as 0.71, $u_{Recovery}$ as 0.45, and $u_{Repeatability}$ as 0.51. $u_{Combined}$ was calculated by taking the square of the sum of the squares of all parameters and was found to be 0.99. $u_{Expanded}$ was calculated with $u_{Combined}$ and was found to be 1.98.

It was found that the highest uncertainty comes from the $u_{Linearity}$ parameter. Possible sources of measurement uncertainty are mostly equipment effects, environmental conditions, and random effects. Taking into consideration equipment effects, uncertainty of linearity can be caused by pipettes used during preparation of the samples. Although calibrated pipettes were used, there was the disadvantage of working with small volumes, and hence the contribution of random errors due to pipetting to measurement uncertainty is large.

Since no ruggedness study was conducted, we do not have data to evaluate analyzer-induced errors at this point. However, analyst-induced variations may also have affected the results. A column oven was not used, and it is possible that there were changes in ambient temperature based on the days of the study. In this case, a minimal variation due to volume change may have created uncertainty.

3.4. Application of the RP-HPLC Method to Determine the Encapsulation Efficiency and Release Profile of the PAA–PLGA Formulation

As mentioned in the experimental section, the encapsulation efficiency of the PAA–PLGA formulation was determined using an indirect method to determine the remaining amount of talazoparib

in the supernatant after encapsulation. Based on our results, the encapsulation efficiency of talazoparib was found to be 65.17% ± 0.50 (n=6). The release profile of the PAA–PLGA formulation is given in Figure 2. As seen from the figure, the release of talazoparib was achieved at 40% within less than 5 h and remained stable until 25 h.

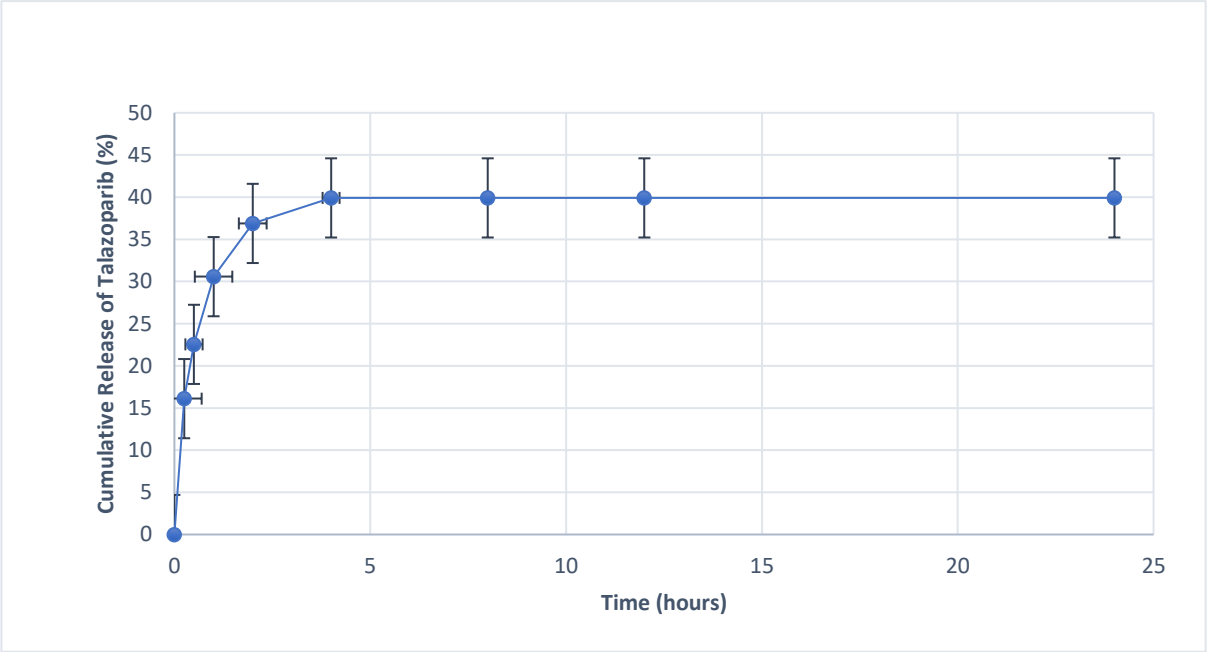

Figure 2. Percent cumulative release versus time of the PAA–PLGA formulation talazoparib

Quality control is a system of validated procedures in which many samples, including active pharmaceutical ingredients and finished products, are analyzed using standard or validated analytical methods. Analytical methods used in the analysis of active pharmaceutical ingredients or final products in the pharmaceutical industry can be methods registered in pharmacopoeias, as well as methods developed by the company itself. For this reason, pharmaceutical analysis attracts the attention of analysts and researchers [15]. Drug delivery systems are innovative systems that enable the active substance to reach the targeted area in a therapeutic dose and without losing its therapeutic effect to provide an effective drug treatment. Due to the inadequacy of traditional drug formulations in treatment, studies on nanoparticular drug delivery systems have attracted increased interest. To eliminate problems such as side effects, multi-drug resistance, and oral bioavailability, especially seen in chemotherapy and affecting the success of the treatment, the approach of preparing anticancer drugs with polymeric nanoparticles has been adopted, and innovative drug formulations have begun to be created in this area [16,17]. In the present study, the encapsulation efficiency value of the PAA–PLGA formulation, developed to overcome the known solubility problem of talazoparib, a PARP inhibitor [18], was greater than 65%. In this case, the formulation we developed was successful in encapsulating the talazoparib molecule to increase its solubility, which is the first problem to be overcome when targeting cancer cells. When the release profile is examined, the release of talazoparib for the developed formulation was achieved at 40% within less than 5 h and remained stable until 25 h. In this case, it appears possible to provide long-term release of talazoparib for the targeted cancer cell with the developed formulation. The RP-HPLC method developed for the examined encapsulation efficiency and release profile is a selective method that works in the range of 0.1 to 12.5 µg/mL and allows selective analysis of the components coming from the nanoformulation without interfering with the active substance peak. When the literature is examined, no HPLC method has been developed specifically for the analysis of the active ingredient talazoparib from pharmaceutical preparations, and the method developed in our study can perform analysis in less than 8 min, which will allow its use in future studies. Considering all these aspects, the study was carried out to contribute to

cancer research by presenting a formulation developed for the targeted and long-term release of talazoparib against cancer cells and an RP-HPLC method that enables analysis of this formulation.

4. Conclusions

Talazoparib is among the newly developed PARP inhibitors, a promising drug group for different diseases, including various types of cancer. There are currently tablet and capsule formulations on the market. Although talazoparib was originally designed to be used in the treatment of breast cancer, its effects on other cancers, such as brain tumors, are now being studied. This ingredient is not chemotherapeutic and kills tumor cells by using damage already existing in the cells. Considering the physicochemical properties of the active substance, it is clear that a major factor influencing the efficacy of commercial formulations is solubility, as reported in previous studies. To solve the solubility problem of this unique compound, we developed a PLGA nanoparticle system. The low solubility of talazoparib is a barrier that needs to be overcome in the phases of drug development. This can be done by nanoparticle systems. PLGA is a biodegradable and biocompatible polymer. It is often used in the development of nanoparticles. That is why this system that has been designed is thought to contribute to the literature in terms of both substance and formulation. Nowadays, in the literature, the number of nanoparticles containing talazoparib is very limited. However, as mentioned in the introduction section, the published analytical methods to determine talazoparib were specific to biological samples, and there was no RP-HPLC method reported previously for analyses of formulations. In the present study, a PLGA nanoparticle system for the controlled release of talazoparib was prepared, and the basic characteristics of the developed formulation were analyzed using RP-HPLC. The total analysis time was less than 8 min in an isocratic elution, while the flow rate was 1.0 mL/min. The developed RP-HPLC method determined the basic characteristics of the PLGA nanoparticle system, and the analytical methodology could be adapted for further studies to determine talazoparib in pharmaceutical formulations and novel drug release systems.

Acknowledgments

This study was supported by a grant (220S765) from the Scientific and Technological Research Council of Türkiye (TÜBİTAK). Beril Tas Topcu is supported by a TÜBİTAK 2244 grant, covering the cooperation between Hacettepe University and Novagenix Bioanalytical Drug R&D Center.

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