Journal of Research in Pharmacy

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Simultaneous determination of albendazole sulfoxide and praziquantel from PLGA nanoparticles and validation of new HPLC method

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Received: 14 May 2022 / Revised: 06 July 2022 / Accepted: 18 July 2022

ABSTRACT: A simple, rapid and reproducible HPLC method has been developed and validated for the quantification of albendazole sulfoxide (ALBSOX) and praziquantel (PRZ), which can coexist in various dosage forms. Chromatographic separation was performed in gradient mode using a mobile phase consisting of an InertSustain® C18 column (150 × 4.6 mm, 5 µm) and acetonitrile: water (v/v) at a flow rate of 1.0 mL/min. The active substance peaks were well separated and detected by a DAD detector at 217 nm. The HPLC method was linear for ALBSOX and PRZ in the concentration range of 0.1-50 µg/mL. Limit of detection (LOD) was found to be 0.01 µg/mL for ALBSOX and 0.009 µg/mL for PRZ. The limit of quantification (LOQ) was found to be 0.03 µg/mL for ALBSOX and 0.027 µg/mL for PRZ. The developed HPLC method was validated based on ICH guidelines for specificity, linearity, system suitability, precision and accuracy. The method was applied for simultaneous quantification and characterization studies of ALBSOX and PRA in PLGA nanoparticles.

KEYWORDS: Albendazole sulfoxide; HPLC method; nanoparticle; praziquantel; validation.

1. INTRODUCTION

Hydatid cyst disease in humans is a zoonotic disease that has spread almost all over the world, resulting from oral ingestion of metacestode larvae of Echinococcus granulosus sensu lato [1-3]. Praziquantel (PRZ), an anthelmintic drug with weak activity against E. granulosus alone, increases the bioavailability of albendazole sulfoxide (ALBSOX), the active metabolite of albendazole [4-6]. In addition, the combination of praziquantel and albendazole sulfoxide is more effective than albendazole alone in patients with intra-abdominal hydatid cysts [4, 7, 8]. Albendazole sulfoxide causes degeneration in microtubules and microthrixes by blocking the glucose absorption of the parasite, thus initiating cellular autolysis and causing the death of parasite cells [9, 10]. Praziquantel, on the other hand, prevents the glucose uptake of parasite cells and ensures depletion of glycogen stores and also accelerates phagocytosis by increasing the passage of calcium into the parasite cells [9, 11]. The chemical structures of albendazole sulfoxide and praziquantel are shown in Figure 1.

How to cite this article: Gültekin Y, Öztürk N, Filazi A, Deniz A, Korkmaz Ç, Pezik E, Barın G, Vural İ. Simultaneous determination of albendazole sulfoxide and praziquantel from PLGA nanoparticles and validation of new HPLC method. J Res Pharm. 2022; 26(6): 1608-1618.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.252

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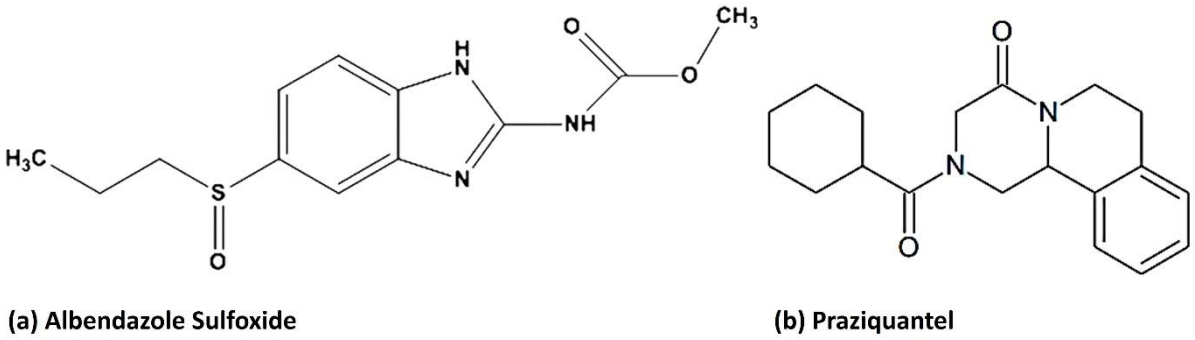

(a) Albendazole Sulfoxide (b) Praziquantel

Figure 1. Chemical structure of albendazole sulfoxide (a) and praziquantel (b)

The success of the chemotherapeutic treatment of hydatid disease depends on the exposure of the protoscolex of the germinal layer and the inner part of the hydatid cyst to the chemotherapeutic in sufficient concentrations for a sufficient period of time and the passage of drugs through the cyst wall [1-3]. To achieve this goal, we have developed extended release PLGA nanoparticle formulations loaded with albendazole sulfoxide and praziquantel.

In the literature, there are many methods developed for HPLC analysis of albendazole sulfoxide [12-16] alone and praziquantel [17-19] alone. In addition, several HPLC analysis methods have been developed for the simultaneous determination of albendazole and praziquantel from pharmaceutical products or biological samples [20-23]. However, there is only one study developed for the simultaneous determination of albendazole sulfoxide and praziquantel [24]. This method, developed by Bonato et al., was developed with high-performance liquid chromatography-electrospray mass spectrometry (LC–MS–MS).

The suitability of the method in the development of a new HPLC method is based on validation. Linearity, specificity, precision, accuracy, limit of quantity (LOQ) and limit of detection (LOD) are the key elements required for validation of a new method, according to the ICH guidelines [25, 26].

Recently, various nanoparticulate formulations of albendazole sulfoxide and praziquantel have been developed for the treatment of hydatid cysts [1, 27-31]. Considering the combined use of albendazole sulfoxide and praziquantel, it is imperative to develop a simple and rapid analytical method for the simultaneous determination of albendazole sulfoxide and praziquantel from nanoparticles. Such methods to be developed can be easily adapted for characterization studies of nanoparticles, quality control studies and similar studies. The aim of this study is to develop and validate a sensitive, simple, fast, economical and single column HPLC method for the simultaneous determination of albendazole sulfoxide and praziquantel from PLGA nanoparticles in the same chromatographic study.

2. RESULTS AND DISCUSSION

2.1. Choosing the appropriate UV wavelength

Using UV spectroscopy, the appropriate wavelength required for simultaneous detection of ALBSOX and PRZ was scanned in the 190-400 nm range. The UV spectra of the two drugs are shown in Figure 2. The maximum absorbance for ALBSOX and PRZ occurred at 228 and 208 nm, respectively. The wavelength of 217 nm was chosen for the simultaneous detection of two drugs, and an acceptable response was obtained at this wavelength for the detection of drugs individually or in combination.

http://dx.doi.org/10.29228/jrp.252
J Res Pharm 2022; 26(6): 1608-1618

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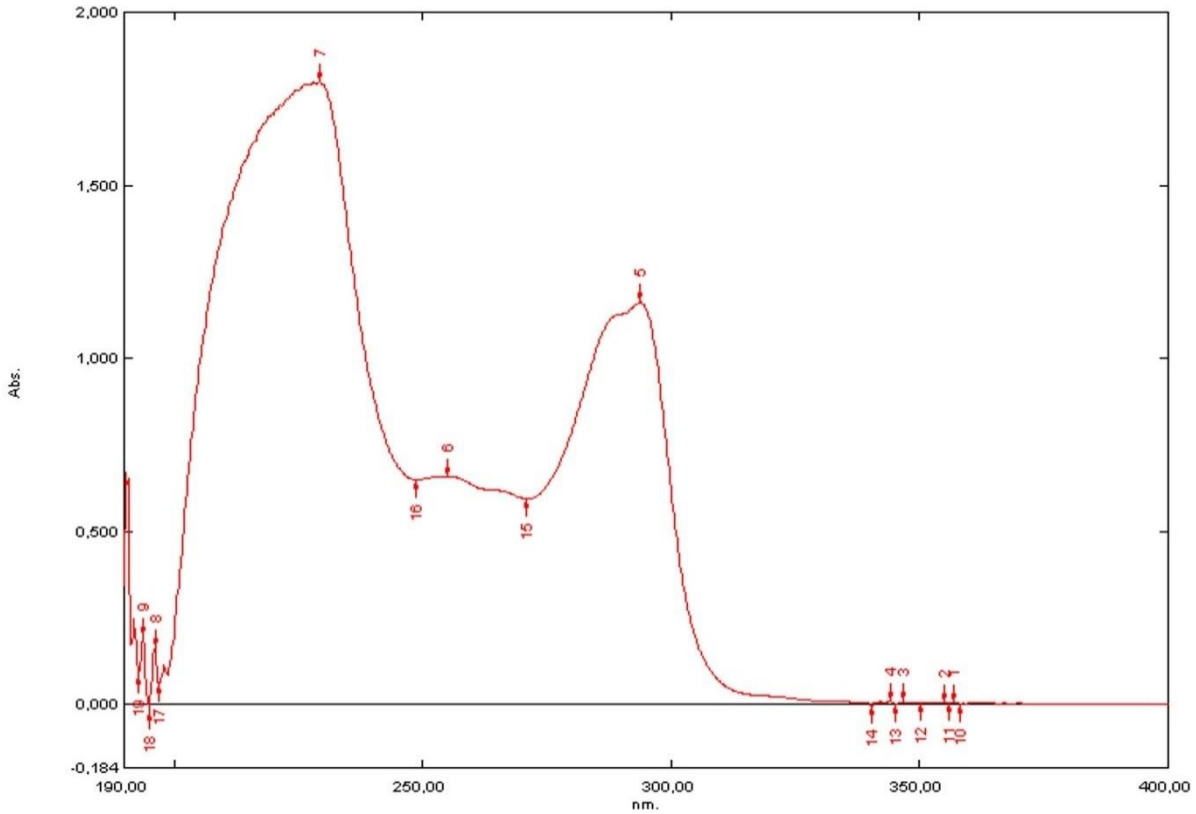
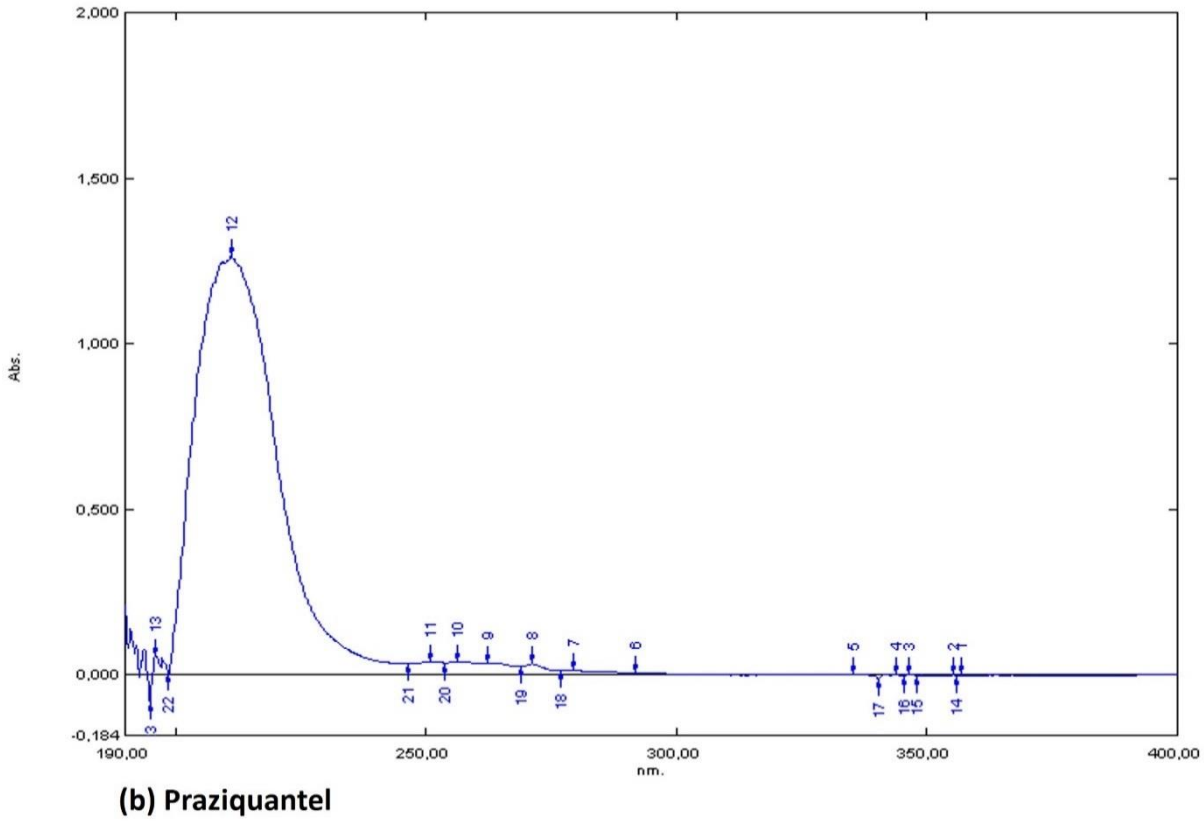


(a) Albendazole sulfoxide



(b) Praziquantel

Figure 2. UV spectra of albendazole sulfoxide (a) and praziquantel (b)

http://dx.doi.org/10.29228/jrp.252
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2.2. Method development and optimization of chromatographic conditions

The development of the method is based on experience gained from HPLC methods previously developed for the analysis of ALBSOX and PRZ alone [15, 17]. A reversed-phase InertSustain® C18 column (150 x 4.6 mm, 5 µm) was used in this study, as previous studies suggested using a C18 reversed-phase column. The mobile phase compositions were varied for the separation of combined ALBSOX and PRZ. A double or triple mobile phase consisting of several acetonitrile, methanol, water and buffer with different ratios was tried and it was decided to use water: acetonitrile as a result. Although initially the isocratic separation mode was tried, it was insufficient for good peak separation and therefore the gradient mode was chosen to provide good and short separation of the peaks of the analytes. The optimized gradient program was developed by changing the water: acetonitrile ratios to keep the overall run time to a minimum. With the final optimized gradient program, a two-stage gradient program consisting of water: acetonitrile with a total run time of 15 minutes was obtained. The mean retention time of ALBSOX and PRZ is 3.7 and 8.8 minutes, respectively. The resolution must be at least 1.5 for any two consecutive peaks to be completely separated [36, 37]. The resolution calculated between the peaks of ALBSOX and PRZ was found to be greater than 1.5, indicating that the resolution is sufficient.

2.3. HPLC method validation

2.3.1. Specificity

The complete separation of ALBSOX and PRZ and the absence of any interference with the peaks in the presence of placebo shows us the selectivity of the method. The resulting peaks were sharp and well separated from the baseline, and the excipients in placebo did not interfere with the peaks, no interference was observed in the retention times of samples ALBSOX and PRZ, proving that the method was specific. Chromatograms obtained for placebo and sample are shown in Figure 3.

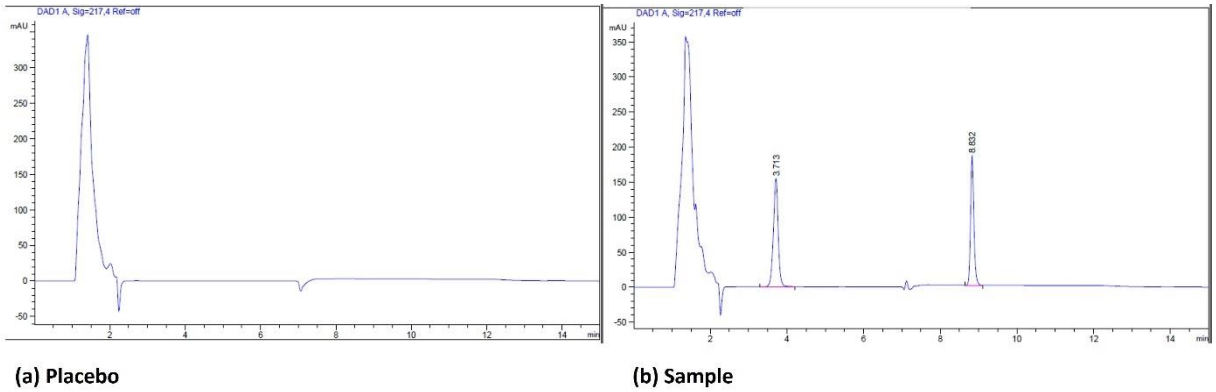

Figure 3. Placebo (a) and sample (b) chromatograms

2.3.2. Linearity

Calibration curves were generated for both ALBSOX and PRZ at seven different concentrations between 0.1-50 µg/mL. Linearity was evaluated by calculating linear regression and correlation coefficient using the graph between peak area and concentration. The linear regression equations for ALBSOX and PRZ were found to be y = 69.793x + 3.3856 and y = 59.297x + 2.2695, respectively. Correlation coefficients were found to be r^2 = 0.9996 for ALBSOX and r^2 = 0.9997 for PRZ, and as a result, an acceptable correlation was found between peak area ratios and drug concentrations. The calibration curve of ALBSOX and PRZ is shown in Figure 4.

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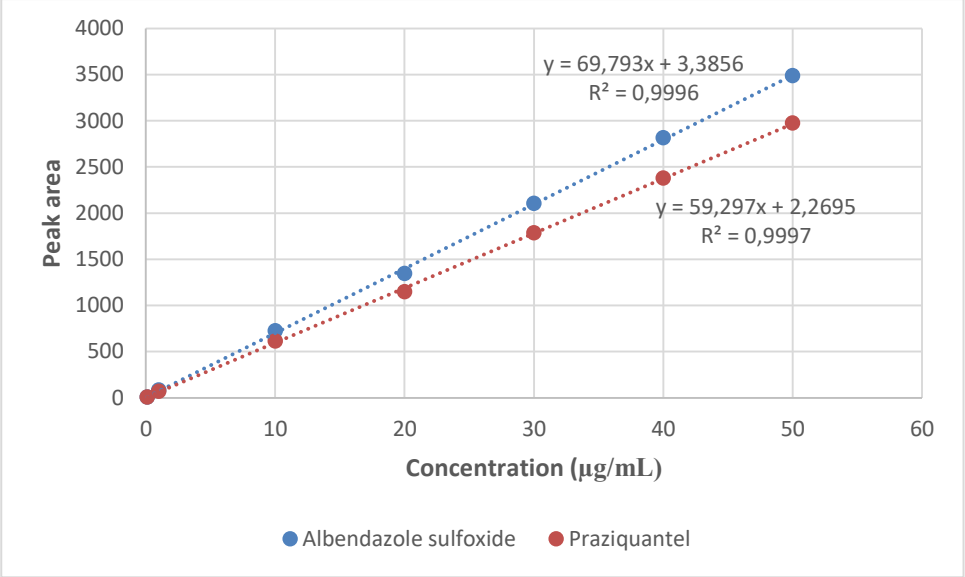

Figure 4. Calibration curve of ALBSOX and PRZ

2.3.3. System suitability

According to the results obtained from 6 repetitive injections given in Table 1, the RSD% values of the peak area and retention time are below 2% and indicate the suitability of the system.

Table 1. System compatibility study results (acceptance limit RSD% <2), (ALBSOX and PRZ 30 µg/ml)

	ALBSOX		PRZ	
	Peak area	Retention time (min)	Peak area	Retention time (min)
n1	2065.05	3.74	1749.80	8.87
n2	2069.55	3.78	1768.11	8.88
n3	2081.63	3.74	1798.97	8.84
n4	2107.95	3.67	1795.38	8.81
n5	2112.84	3.66	1796.32	8.81
n6	2114.98	3.67	1795.33	8.81
Mean	2092.00	3.71	1783.98	8.83
SD[a]	22.60	0.04	20.27	0.03
RSD[b]%	1.08	1.34	1.13	0.36

[a]Standard deviation,

[b]Relative standard deviation.

2.3.4. Precision

As a result of the determination of 6 independent combined samples of ALBSOX and PRZ, the peak areas were reproducible and precise over 3 days. As can be seen in Table 2, the method has high precision and reproducibility as the %RSD values for both ALBSOX and PRZ are below 0.8% as a result of intraday and interday determinations.

Table 2. Results of method precision study (acceptance limit RSD% <2), (ALBSOX and PRZ 20 µg/mL)

	Day 1		Day 2		Day 3	
	ALBSOX	PRZ	ALBSOX	PRZ	ALBSOX	PRZ
n1	1334.88	1138.30	1320.62	1128.94	1314.59	1122.80
n2	1333.62	1139.81	1322.99	1131.64	1327.61	1135.09
n3	1333.63	1142.18	1321.22	1132.40	1325.50	1133.62
n4	1337.16	1139.96	1320.29	1130.61	1324.85	1132.05
n5	1335.75	1143.54	1322.34	1131.83	1316.83	1126.11
n6	1352.26	1154.73	1323.40	1130.61	1343.05	1148.21
Mean	1337.88	1143.08	1321.81	1131.05	1325.40	1132.98
SD[a]	7.17	6.00	1.28	1.23	10.06	8.81
RSD[b]%	0.53	0.52	0.09	0.10	0.75	0.77

[a]Standard deviation,

[b]Relative standard deviation.

2.3.5. Accuracy

The accuracy of the proposed method was evaluated by determining the recovery values in triplicate at three different concentrations of 10, 20, and 30 µg/mL, 50%, 100%, and 150% of the combined ALBSOX and PRZ sample concentration (20 µg/mL). The recovery percentage was found in the range of 98.55-101.55% and 98.67-101.71% for ALBSOX and PRZ, respectively. As seen in Table 3, the results obtained show the accuracy of the method.

Table 3. Accuracy study results of ALBSOX and PRZ (acceptance limit RSD% <2)

Intraday Analysis

Albendazole Sulfoxide					Praziquantel				
Constant amount added (µg/ml)	Peak area	Amount found (µg/ml)	% Recovery	%RSD[a]	Constant amount added (µg/ml)	Peak area	Amount found (µg/ml)	% Recovery	%RSD[a]
10	701,46±2,04	10,00±0,03	100,02±0,29	0,29	10	600,17±1,05	10,08±0,03	100,83±0,25	0,25
20	1393,3±14,20	19,91±0,20	99,57±1,02	1,02	20	1186,96±11,15	19,97±0,18	99,89±0,94	0,94
30	2116,88±6,41	30,28±0,09	100,94±0,30	0,30	30	1802,95±4,10	30,36±0,06	101,22±0,22	0,22

Interday Analysis

Albendazole Sulfoxide					Praziquantel				
Constant amount added (µg/ml)	Peak area	Amount found (µg/ml)	% Recovery	%RSD	Constant amount added (µg/ml)	Peak area	Amount found (µg/ml)	% Recovery	%RSD
10	698,37±7,31	9,96±0,06	99,58±0,60	0,60	10	601,63±3,65	10,11±0,02	101,08±0,19	0,19
20	1379,04±18,40	19,71±0,22	98,55±1,11	1,11	20	1195,42±16,30	20,12±0,24	100,61±1,19	1,19
30	2096,47±9,31	29,98±0,08	99,93±0,26	0,26	30	1776,68±5,64	29,92±0,06	99,73±0,20	0,20

[a]Relative standard deviation.

2.3.6. Stability

Short-term stability studies have shown that the standard and sample solutions are stable when kept on the bench at room temperature for 24 hours. The results of short-term stability studies are shown in Table 4.

Table 4. Stability study results (acceptance limit % <2)

	Standard Solution		Sample Solution	
	ALBSOX	PRZ	ALBSOX	PRZ
Beginning (area)	1353.84	1169.46	1334.70	1137.90
After 24 hours (area)	1346.16	1148.54	1351.02	1142.53
Stability (%)	100.57	101.82	98.79	99.59

2.3.7. Robustness and ruggedness

The result of the robustness and ruggedness of the method is summarized in Table 5. After deliberate variations in mobile phase flow rate and temperature, the robustness of the method was checked, the RSDs of the analyzed samples were found to be less than 2%, and it was determined that the changes in operational parameters did not cause significant changes in the performance of the chromatographic system. In the sample analyzes performed by different analysts on different days, the RSD values were found to be less than 2% and the ruggedness of the method was determined to be acceptable.

Table 5. Summary of robustness and ruggedness data

		Albendazole Sulphoxide		Praziquantel	
Parameter	Modification	% Recovery	RSD[a]	% Recovery	RSD[a]
Flow rate (ml/min)	0,9	98,3	1,1	98,9	1,3
	1	100,2	0,6	100,5	0,8
	1,1	100,9	0,8	101,1	1,1
Temperature (°C)	22	100,9	0,8	101,3	1,5
	25	99,7	0,4	99,9	0,9
	28	98,5	0,5	98,8	1,7
Analyst	Analyst 1	99,7	0,3	100,1	0,5
	Analyst 2	98,3	1,2	98,5	1,6
Different days	Day 1	99,6	0,7	100,1	0,6
	Day 2	99,9	0,9	100,3	0,7

[a]Relative standard deviation.

3. CONCLUSION

The present method differs from previously reported methods for separate and combined analysis of ALBSOX and PRZ in terms of mobile phase and chromatographic conditions. Moreover, all previously developed methods were designed for the analysis of ALBSOX and PRZ from biological fluids or tablets. However, in this study, a sensitive, simple, rapid, economical, and single-column gradient HPLC method for the simultaneous analysis of ALBSOX and PRZ from PLGA nanoparticle formulations was developed and validated for the first time. The developed HPLC method enables simple, accurate and reproducible quantitative analysis for the development, characterization and quality control of ALBSOX and PRZ loaded nanoparticle formulations without any interaction with PLGA nanoparticles as excipients. For these reasons, this developed method is recommended for the simultaneous determination of ALBSOX and PRZ from PLGA nanoparticles.

4. MATERIALS AND METHODS

4.1. Materials

Albendazole sulfoxide and praziquantel analytical standards (purity>99%) were purchased from HPC Standards (Germany). HPLC grade acetonitrile, methanol, and DMSO were purchased from Sigma–Aldrich (St. Louis, USA). A Millipore Milli-Q ultrapure water system (Millipore, Australia) was used to obtain highly pure water.

4.2. Choosing the appropriate UV wavelength

For the simultaneous determination of albendazole sulfoxide and praziquantel, it was necessary to select an appropriate wavelength. The wavelength required for the detection of two active substances was determined by wavelength scanning in the 190-400 nm range using a Shimadzu UV-1800 (Shimadzu, Japan) spectrophotometer [32].

4.3. Chromatographic conditions

An Agilent 1200 series system (Wilmington, DE, USA) consisting of an Agilent 1200 G1322A deaerator, Agilent 1200 G1311A quad pump, Agilent 1200 G1329A autosampler, Agilent 1200 G1316A thermostated column chamber and Agilent 1200 G1315D DAD detector was used to develop the HPLC method. Separation was performed using an InertSustain® C18 column (150 x 4.6 mm, 5 µm) (GL Sciences / Japan). The mobile phase system consisting of water and acetonitrile and bimodal gradient elution were applied at a flow rate of 1 mL/min by changing the solvent ratios as shown in Table 6. 20 µl of sample was injected into the HPLC system and the column temperature was set to 25 °C.

Table 6. Gradient program of the HPLC method

Time, min	Water%	Acetonitrile %
0	75	25
4	75	25
5	40	60
10	40	60
11	75	25
13	75	25

4.4. Preparation of solutions

4.4.1. Preparation of standard solutions of albendazole sulfoxide and praziquantel

25 mg of each of ALBSOX and PRZ were accurately and precisely weighed and transferred to 25 mL flasks separately and dissolved by adding 20 mL of methanol, then made up to volume with methanol. Appropriate dilutions of these stock solutions were prepared using methanol to prepare solutions of known concentrations. Standard and stock solutions were stored at 4 °C, protected from light, in order to maintain their stability.

4.4.2. Preparation of albendazole sulfoxide and praziquantel standard solutions in PLGA nanoparticle matrix

25 mg ALBSOX and 25 mg PRZ samples were mixed with PLGA nanoparticle matrix components in appropriate proportions. Then, 1 mL of DMSO was added to the mixture, made up to 25 mL with methanol, and vortexed for 10 minutes and sonicated. The resulting solution was centrifuged at 4000 rpm for 5 minutes and the supernatant was taken and used in the studies.

4.4.3. Test sample preparation

PLGA nanoparticle formulations containing 25 mg of ALBSOX and PRZ were weighed and the nanoparticles were fragmented using 1 mL of DMSO. Then methanol was added up 25 mL and mixture was

vortexed and centrifuged. The obtained supernatant was diluted with methanol at appropriate ratios and filtered through a 0.45 µm filter.

4.5. HPLC method validation

4.5.1. Specificity

The specificity of the analytical method was confirmed by analysis of a solution containing a certain amount of PLGA nanoparticle excipients added 20 µg/mL ALBSOX and PRZ. The ability of the method to separate the nanoparticle excipients in the sample from ALBSOX and PRZ was determined by evaluating the resolution between the peaks corresponding to the various substances. The specificity of the proposed HPLC method for the determination of ALBSOX and PRZ was established by injecting the placebo matrix and test sample into the HPLC system [26, 33].

4.5.2. Linearity

A calibration curve was created by plotting the peak area response obtained against the concentration of ALBSOX and PRZ. For this, standard solutions containing 0.1, 1, 10, 20, 30, 40, 50 µg/mL ALBSOX and PRZ were used. Standard solutions were prepared in 4 replicates and injected into a 20 µL HPLC column. Linearity was evaluated using the graph between peak area and concentration, linear regression and correlation coefficient [26, 32].

4.5.3. System suitability

To determine the suitability of the system, 20 µg/mL ALBSOX and PRZ were used as standard solutions. Six replicate injections of this standard solution were analyzed by HPLC, and the peak area and retention time were evaluated as acceptance criteria [34].

4.5.4. Precision

Method precision was assessed by injecting 6 independent combined samples of ALBSOX and PRZ on the same day under the same operating conditions and the relative standard deviation (RSD) % was calculated. Intermediate precision was examined by comparing the results of 6 independent determinations on 3 different days [26, 33].

4.5.5. Accuracy

The accuracy of the method was evaluated by determining the recovery values. Accuracy means the closeness of the sample to the reference value. To determine accuracy, the combined ALBSOX and PRZ were analyzed in triplicate at three different concentrations of 10, 20, and 30 µg/mL, 50%, 100%, and 150% of the sample concentration (20 µg/mL) [25, 26].

4.5.6. Limit of detection and limit of quantitation

Limits of detection (LOD) and quantification (LOQ) for combined ALBSOX and PRZ were estimated using the signal-to-noise ratio. The lowest concentration level equal to a peak area of three times the signal-to-noise ratio was set as the detection limit. The lowest concentration level equal to a peak area of ten times the signal-to-noise ratio was determined as the quantitation limit [26, 34, 35].

4.5.7. Stability

Short-term stability was evaluated using the peak areas obtained by analyzing standard solutions and sample solutions kept at room temperature for 24 hours [34].

4.5.8. Robustness and ruggedness

The robustness of the HPLC method was determined by analyzing samples under different conditions as a result of deliberate small changes in temperature and mobile phase flow rate. The effect on retention time and peak parameters was examined. The ruggedness of the HPLC method was determined by sample analyzes performed on different days by two different analysts without changing the method conditions [32, 33, 38].

Acknowledgements: This study was supported by Hacettepe University Scientific Research Projects Coordination [Unit with grant number TSA-2021-19166].

Author contributions: Concept – Y.G., İ.V., A.F., A.D., N.Ö.; Design – Y.G., İ.V., A.F., N.Ö.; Supervision – İ.V., A.F., N.Ö.; Resources – Y.G., İ.V., A.F.; Materials – Y.G., İ.V., A.F.; Data Collection and/or Processing – Y.G., İ.V.; Analysis and/or Interpretation – A.F., İ.V., A.D., Y.G., N.Ö.; Literature Search – Y.G., Ç.K., E.P., G.B.; Writing – Y.G.; Critical Reviews – A.D., N.Ö., A.F., İ.V.

Conflict of interest statement: The authors declared no conflict of interest.

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