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Original Article

A Unified and Isocratic RP-HPLC-UV Method for Azithromycin Determination Across Multiple Pharmaceutical Dosage Forms

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Abstract

Analysis of azithromycin (AZT) by reversed-phase high-performance liquid chromatography (RP-HPLC) is often limited by peak tailing caused by silanol interactions and by reliance on dosage-form-specific Pharmacopeia methods. A unified and column-compatible RP-HPLC-UV method has been developed for the determination of AZT in various pharmaceutical dosage forms. Chromatographic separation was performed on a C18 column using a mobile phase of 0.02% triethylamine (TEA) in 10 mM phosphate buffer (pH 7.0 ± 0.05) and acetonitrile (60:40, v/v) under isocratic conditions with UV detection at 210 nm. Validation according to ICH Q2(R2) demonstrated strong linearity (R^2 = 0.9985) over the range of 0.80–1.20 mg/mL, with %residuals within ±2%. Accuracy ranged from 100.41% to 101.00%, and precision (repeatability and intermediate precision) was confirmed by %RSD < 1%. The method exhibited acceptable robustness under the evaluated conditions; however, retention was sensitive to variations in mobile phase pH, highlighting the importance of maintaining strict pH control during routine analysis. Application to commercial products resulted in assay values of 99.42%–103.65% of label claim, meeting USP and BP specifications. This method provides a streamlined and practical analytical approach for routine quality control of AZT across multiple pharmaceutical dosage forms using a unified chromatographic approach.

Keywords

Azithromycin, RP-HPLC, isocratic method, analytical method validation, quality control

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Introduction

Azithromycin (AZT) ($C_{38}H_{72}N_2O_{12}$) is a semi-synthetic macrolide antibiotic with a 15-membered azalide ring, widely prescribed to treat bacterial infections, including those of the respiratory tract and skin.[1–5] The physicochemical properties of AZT include a molecular weight of 749.0 g/mol, solubility in ethanol and dimethyl sulfoxide (DMSO), minimal solubility in water (0.514 g/L), a pKa between 8.50 and 8.74, and low UV absorbance.[6] Its extensive use across multiple pharmaceutical dosage forms, such as tablets, capsules, and oral suspensions, requires reliable, robust analytical methods to ensure quality control and regulatory compliance.

The analysis of AZT using reversed-phase high-performance liquid chromatography (RP-HPLC) presents several challenges. As a basic compound, AZT interacts with residual silanol groups on silica-based stationary phases, resulting in peak tailing and diminished chromatographic performance.[7–11] In such cases, amine-containing mobile phase modifiers have been reported to reduce peak tailing by masking residual silanol interactions and improving peak symmetry,[11–14] as shown in Figure 1. Depending on the chromatographic conditions, different amine additives, including

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triethanolamine and triethylamine, have been employed in different chromatographic methods. These considerations highlight the importance of selecting appropriate mobile phase modifiers to improve chromatographic performance for basic compounds. Furthermore, complex pharmaceutical matrices and excipient compositions can complicate sample preparation and compromise analytical accuracy.[15–20]

Multiple HPLC methods have been reported for the determination of AZT in pharmaceutical formulations.[21–27] These approaches typically utilize buffer–acetonitrile (ACN) systems under isocratic conditions and have demonstrated satisfactory analytical performance. However, many of these methods require high buffer concentration or extreme pH conditions, involve complex mobile-phase compositions, or are restricted to specific dosage forms, thereby limiting their practicality for routine quality control laboratories.

Pharmacopoeial methods for AZT products vary considerably by dosage form. The United States Pharmacopoeia (USP) version USP44-NF39 assay for tablets employs ultraviolet (UV) detection at 210 nm with an L1 column (octadecylsilane chemically bonded to porous or non-porous silica or ceramic microparticles, C18) and a phosphate buffer–ACN mobile phase containing approximately 33.80 mM phosphate buffer at pH 7.5. In comparison, the USP assay for capsules uses electrochemical detection, a different mobile phase at pH 11.0, and an L-29 (gamma-alumina) column. The method for oral suspension requires a buffer at pH 8.2 and an L1 column.[28–30] Moreover, in the British pharmacopoeia (BP) version 2024, the assay for tablet formulation uses UV detection at 210 nm with octadecylsilyl vinyl polymer column (C18) for chromatography, and a 40% (v/v) solution of 0.67% w/v dipotassium hydrogen orthophosphate (38 mM), adjusted to pH 11.0, in ACN as the mobile phase. For the capsule assay, the chromatographic conditions are the same as for the tablets, with the same detection wavelength and column; the mobile phase is a 40% (v/v) solution of 38 mM dipotassium hydrogen orthophosphate, adjusted to pH 11.0 with 56% w/v solution of potassium hydroxide, in ACN. The oral suspension assay uses an octadecylsilyl vinyl polymer column for chromatography, a detection wavelength of 210 nm, and an isocratic mobile phase consisting of 40% (v/v) dipotassium hydrogen orthophosphate solution adjusted to pH 11.0 with potassium hydroxide and 60% (v/v) acetonitrile.[31–33] These dosage-form-specific protocols increase analytical complexity and may impede routine implementation in quality control laboratories. The use of multiple dosage-form-specific methods in pharmacopoeial procedures may increase analytical workload, instrument setup time, and potential variability between methods.

There is a need to develop a simple, robust, and column-compatible HPLC method applicable to multiple

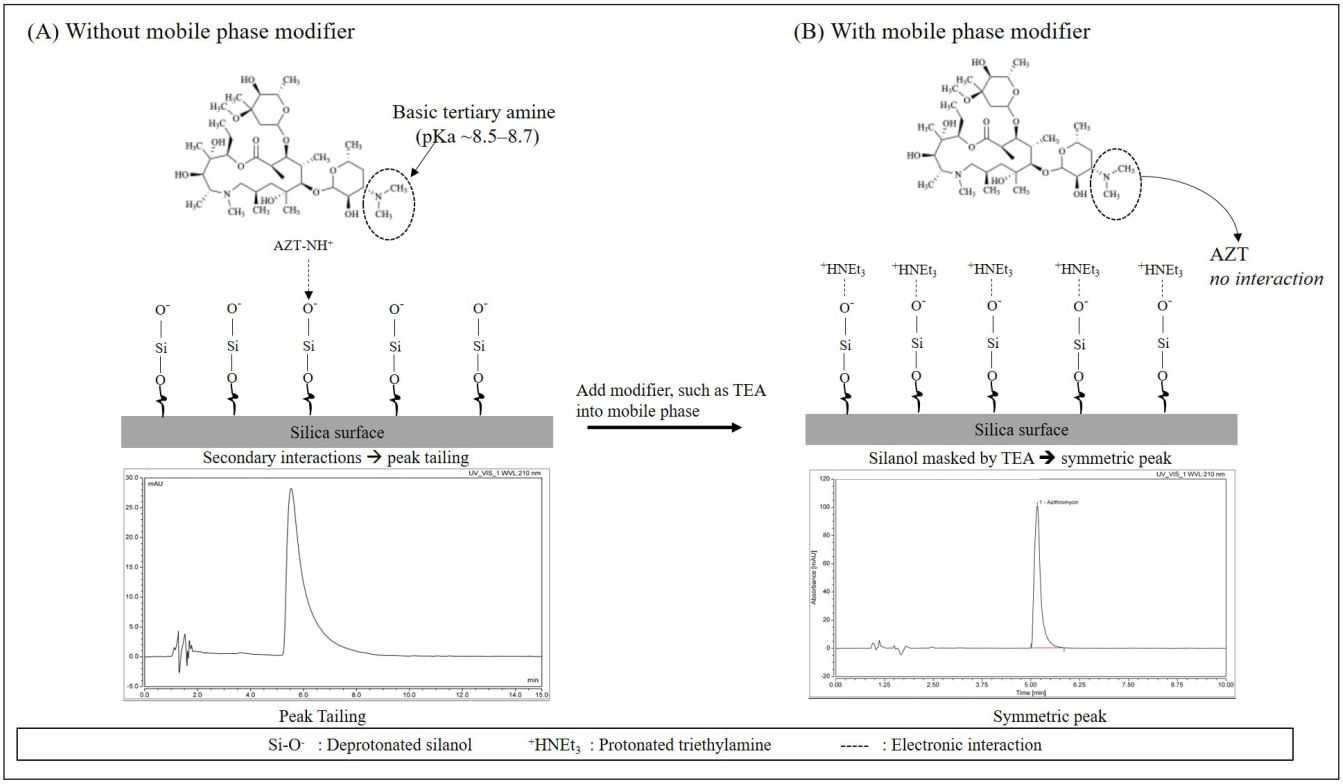

Figure 1. Schematic Illustration of AZT Interaction with Silanol Groups and the Effect of TEA. Secondary Interactions Between Protonated AZT and Silanol Groups Cause Peak Tailing (A), Whereas TEA Masks Silanol Sites, Reducing These Interactions and Improving Peak Symmetry (B).

dosage forms under a single chromatographic condition. Implementing such a unified approach would reduce analytical complexity and enhance efficiency in routine quality control laboratories.

This study developed and validated a unified RP-HPLC-UV method in accordance with ICH Q2(R2) and AOAC International guidelines for the determination of AZT in various pharmaceutical dosage forms.[34,35] The method's applicability was further demonstrated by analyzing selected commercial products available in Thailand.

Materials and Methods

Chemicals and Reagents

In this study, HPLC-grade solvents were used to prepare the mobile phase. The solvents for the HPLC mobile phase were purchased from RCI Labscan. The other solvents and chemicals for standard and sample preparation were analytical grade. The reagents were dipotassium hydrogen phosphate (K_2HPO_4), hydrochloric acid (HCl), methanol (CH_3OH), potassium dihydrogen orthophosphate (KH_2PO_4), TEA, and sodium hydroxide (NaOH). All chemicals were supplied by Acros Organics, Labscan, Merck, Sigma-Aldrich, TCI, and Thermo Fisher Scientific. The excipients used to prepare the synthetic formulations of film-coated tablets, capsules, dry syrup, and dry suspension included acacia, ascorbic acid, citric acid, corn starch, glycerin, hydroxypropyl methylcellulose (HPMC), lactose monohydrate, magnesium stearate, mannitol, methyl paraben, methylcellulose, orange flavor, polyethylene glycol 400 (PEG-400), polyethylene glycol 4000 (PEG-4000), polyvinylpyrrolidone (PVP), propylene glycol, propyl paraben, saccharin, span 80, sodium benzoate, sodium citrate, sodium starch glycolate, sorbitol, stearic acid, sucrose, talcum, tartrazine, titanium dioxide, tween 80, and xanthan gum. All excipients used in this study were pharmaceutical grade and were obtained from authorized distributors in Thailand (P.C. Drug Center, Co., Ltd., and S. Tong Chemicals Co., Ltd.), where the materials are imported and repackaged prior to distribution. The AZT working standard used in this study was kindly supplied by Siam Pharmaceutical Co., Ltd., with a purity of 95.5%.

Commercial AZT pharmaceutical products in various dosage forms were purchased from local markets in Nakhon Si Thammarat, Thailand, for method validation and application. The products included tablets 250 mg/tablet (Zithromax®, Pfizer, Thailand), capsules 250 mg/capsule (Atozin®, Milimed Co., Ltd., Thailand; Floctil® 250, Unison Laboratories Co., Ltd., Thailand; Azithro®, M & H Manufacturing Co., Ltd., Thailand), dry syrup 200 mg/5 mL (Manazith®, T. Man Pharma Co., Ltd., Thailand), and dry suspensions 200 mg/5 mL (Zithromax®, Pfizer, Thailand; Onzet®, M & H Manufacturing Co., Ltd., Thailand; Floctil® 200, Unison Laboratories Co., Ltd., Thailand). All products were confirmed to be within their shelf life at the time of analysis.

Instruments

The HPLC system (Thermo Scientific Vanquish Analytical Purification LC, Waltham, MA, USA), coupled with a Vanquish photodiode array (PDA) detector, was used for the analysis. Data were acquired using Thermo Scientific Chromeleon Chromatography Data System (CDS). Chromatographic separation was achieved with an Agilent ZORBAX Eclipse XDB-C18 column (250 mm × 4.6 mm, 5 µm) with a guard column Agilent Eclipse XDB-C18 (12.5 mm × 4.6 mm, 5 µm). Sample weighing was performed using an analytical balance (Mettler Toledo, Model ME204T, Changzhou, China). The pH of the mobile phase was measured with a pH meter (Mettler Toledo, Model SD20Basic, Changzhou, China).

Chromatographic Conditions

Chromatographic analysis was performed using a mobile phase of 0.02% TEA in 10 mM phosphate buffer (pH 7.0 ± 0.05) and ACN (60:40, v/v) under isocratic conditions. The column temperature was maintained at 30°C, and the mobile phase flow rate was set at 2.0 mL/min. For each run, 40 µL of the sample was injected into the HPLC system. The mobile phase was filtered through a 0.45 µm nylon membrane filter (Thermo Scientific, Waltham, MA, USA), sonicated, and degassed before use. All samples were filtered before injection using a 0.45 µm nylon syringe filter (Jiangsu Green Union Science Instrument Co., Ltd., China). The PDA detector was set to 210 nm to detect AZT in the column effluent, as illustrated in Figure 2.

Buffer Preparation

The buffer for the mobile phase was 10 mM phosphate buffer, pH 7.0, prepared by dissolving 0.843 g of KH_2PO_4 and 0.664 g of K_2HPO_4 in 800 mL of water. The pH was adjusted to 7.0 using a few drops of phosphoric acid (85%), and then 0.2 mL of TEA was added before the solution was adjusted to 1000 mL. The solution was filtered through a 0.45 µm nylon membrane filter and degassed prior to use as the mobile phase in HPLC analysis.

Preparation of Standard Stock Solution

The working standard of AZT 261.78 mg was added to 50 mL in a volumetric flask. The AZT working standard was a qualified secondary reference standard standardized against a

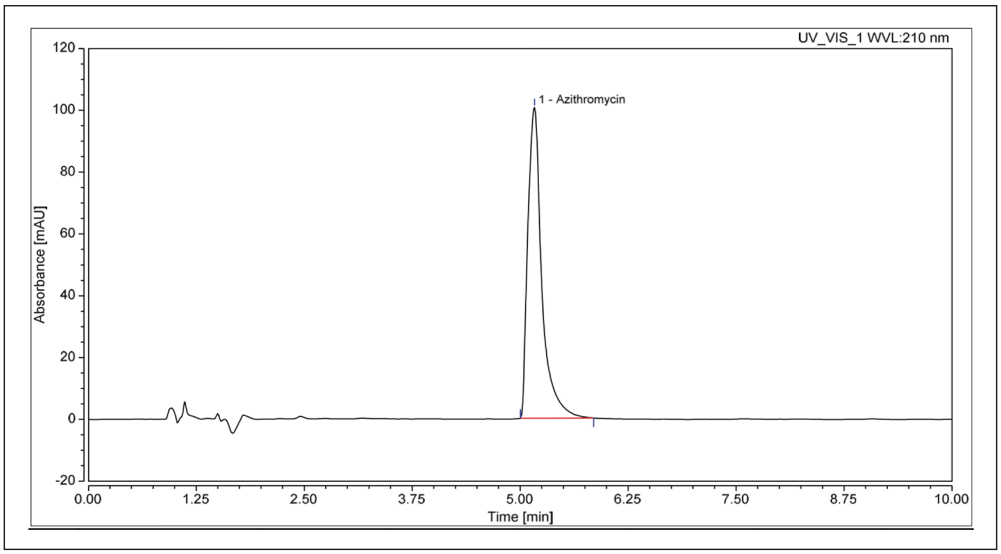

Figure 2. The Optimized Chromatogram of the AZT Standard. The Chromatographic Conditions Were 10 mM Phosphate Buffer With 0.02% TEA:ACN (60:40), a Column Temperature of 30°C, a Flow Rate of 2.0 mL/min, and a Detection Wavelength of 210 nm.

primary reference standard. All standard concentrations were calculated using the certified purity value of 95.5% (w/w). The flask was then filled with 25 mL of diluent (ACN and water (1:1, v/v)), and sonicated for 15 min or until the standard dissolved. The diluent was added to the volumetric flask to volume. The nominal stock solution concentration was 5 mg/mL. All subsequent standard concentrations used for calibration and validation were calculated after correction for the certified purity of the AZT working standard (95.5%, w/w). The standard stock solution was stored in the refrigerator at 2°C–4°C until use.

Analytical Method Development

The method development process for HPLC analysis of AZT involved optimizing various parameters while keeping specific constants. Mobile phase composition (solvent systems and ratios), column oven temperature, and flow rate were adjusted.

Analytical Method Validation According to ICH Q2(R2)

According to ICH Q2(R2)

Validation of analytical procedures guidelines, the developed and optimized RP-HPLC analytical method of AZT was validated.[34]

System Suitability

According to the AZT tablets, capsules and suspension monograph of USP 44-NF39, system suitability for the HPLC analytical method is assessed by performing five replicates of a 1 mg/mL AZT standard solution. The developed method checks the tailing factor (T), the theoretical plate (N) and the relative standard deviation (RSD). The retention time (Rt) is used to compare the standard and the AZT sample.

Carryover was assessed during the analytical sequence by injecting a diluent blank after every 10 injections. The diluent blank comprised the same ACN–water (1:1, v/v) solution used for preparing standards and sample solutions. The lack of detectable peaks at the retention time of AZT in all blank chromatograms indicated negligible carryover.

Specificity and Selectivity

Specificity was determined by comparing standard AZT, excipients (film-coated tablets, capsules, dry syrup, and dry suspension), and blank interference during sample preparation to assess retention time.

Placebo solutions were prepared to evaluate method specificity by simulating the sample preparation procedures for each dosage form. For film-coated tablets and capsules, excipients corresponding to each formulation were accurately weighed and homogenized by grinding in a mortar and pestle to replicate the comminution of pooled tablets and the mixing of capsule contents. The resulting placebo mixtures were processed using the same sample-preparation procedure as that used for the corresponding pharmaceutical products. For dry syrup and dry suspension formulations, excipients were similarly homogenized by grinding and subsequently reconstituted with water according to the same dilution procedure used for the corresponding products. The reconstituted placebo samples were then analyzed identically to the test samples.

Linearity

Three sets of injections were conducted using AZT standard solutions at concentrations ranging from 0.8 to 1.2 mg/mL to

assess linearity. The calibration curve encompassed the entire concentration range. The relationship between peak area (y) and concentration (x) was evaluated using a regression model.

Accuracy

The accuracy of the proposed method was evaluated using the standard addition method at three concentration levels corresponding to 80%, 100%, and 120% of the target concentration (0.80, 1.00, and 1.20 mg/mL). Six replicate determinations were performed at each concentration level, and percentage recovery was calculated to assess method accuracy. The samples were prepared using AZT film-coated tablets available in Thailand, and the concentration of the blank placebo solution was analyzed following the USP44-NF39[28] method: a blank placebo at a final concentration of 0.5 mg/mL and spiked standards at final concentrations of 0.80, 1.00, and 1.20 mg/mL. RSD and recovery percentages were calculated.

Precision (Repeatability and Intermediate)

The precision was evaluated by injecting spike standard samples at concentrations of 80%, 100%, and 120% of 1 mg/mL in six replicates, assessing both intra-day (repeatability) and inter-day (intermediate precision) variability. RSD from recovery percentages was calculated.

LOD and LOQ

Limit of detection (LOD) and limit of quantitation (LOQ) were estimated according to ICH Q2(R2) guidelines[34] using the standard deviation of the response (σ) and the slope of the calibration curve (S), according to the following equations:

$$\mathrm{LOD} = 3.3\sigma/S$$

$$\mathrm{LOQ} = 10\sigma/S$$

Robustness

The robustness of the developed method was evaluated by systematically varying key chromatographic parameters around the optimized conditions. The effect of pH was assessed by adjusting the buffer from 7.0 to 6.8 and 7.2, while the column temperature was varied from 30°C to 29°C and 31°C. The flow rate was changed from 2.0 mL/min to 1.9 and 2.1 mL/min, and the detection wavelength was evaluated by varying it by ±1 nm from 210 nm. The method demonstrated consistent performance under these small, deliberate variations, indicating its reliability under normal operating conditions.

In-use Standard Solution Stability

Standard solution stability assessments were conducted to ensure their integrity throughout the analysis. To assess sample stability, the standard solutions in the HPLC sample chamber were left at room temperature. The standard solution was injected at 0, 12, and 24 h after being kept in the HPLC to demonstrate in-use stability.

Application of the Developed Method

The validated HPLC method was effectively employed to determine and quantify the amounts of AZT in some brands available in Nakhon Si Thammarat, Thailand. The original coated AZT tablets (Zithromax®), three brands of AZT capsules, three brands of AZT dry suspensions (including Zithromax®), and one brand of AZT dry syrup were selected. The sample preparation for each dosage form was performed, followed by the method below.

For the tablet dosage form, use finely powdered film-coated tablets, not less than 20 tablets. To prepare the sample stock solution (5 mg/mL), weigh the fine powder equivalent to 250 mg of AZT into a 50 mL volumetric flask. Add the ACN (25 mL) to the flask, and sonicate for 30 min. After cooling to room temperature, add water to the volume in the 50 mL volumetric flask and mix thoroughly. Pipette 5 mL of the stock sample solution into a 25 mL volumetric flask, then adjust the volume to 25 mL with diluent (ACN:water, 1:1 v/v). The concentration of the sample solution is 1 mg/mL.

For the capsule dosage form, remove, as completely as possible, the contents of not less than 20 capsules. To prepare the sample stock solution (5 mg/mL), weigh the fine powder equivalent to 250 mg of AZT into a 50 mL volumetric flask. Add the ACN (25 mL) to the flask, and sonicate for 30 min. After cooling to room temperature, add water to volume in the 50 mL volumetric flask and mix thoroughly. Pipette 5 mL of the stock sample solution into a 25 mL volumetric flask, then adjust the volume to 25 mL with diluent (ACN:water, 1:1 v/v). The concentration of the sample solution is 1 mg/mL.

For dry oral suspension and dry oral syrup formulations, the products were reconstituted according to the manufacturer's instructions. After reconstitution, the total volume of the suspension was 15 mL, and the labeled concentration was 200 mg/5 mL, corresponding to 40 mg/mL AZT. Therefore, a 6.25 mL aliquot, equivalent to 250 mg of AZT, was transferred into a 50 mL volumetric flask. ACN (25 mL) was added, and the mixture was sonicated for 30 min. The volume was then adjusted to 50 mL with water to obtain a stock sample solution of 5 mg/mL. Subsequently, 5 mL of the stock solution was transferred into a 25 mL volumetric flask and diluted to volume with diluent to obtain the final sample solution at 1 mg/mL.

A 30-min sonication time was selected to ensure complete dissolution and extraction of AZT from all dosage forms. This parameter was applied consistently throughout the study because AZT exhibits relatively low aqueous solubility, and prolonged sonication reduces variability due to incomplete extraction.

All sample solutions were prepared and analyzed within 6 h of preparation to reduce the risk of changes in analyte concentration during storage.

Results and Discussion

Method Development and Optimization

The AZT analysis system using RP-HPLC was developed. All samples were injected at 40 µL and detected at 210 nm. The development of the HPLC method was followed by optimization of the chromatographic factors of temperature, organic concentration, and flow rate. The Rt, N, and T are used to select the optimized chromatographic system conditions.

The previous research for the AZT analytical method development used a C18 column, usually mentioning the pH between 6.8 and 8.0, and most of the research selected the phosphate buffer system (20–100 mM) in the mobile phase.[21–27] The method development started with HPLC conditions using a C18 column, a 60:40 (10 mM phosphate buffer pH 7.0 ± 0.05:ACN) mobile phase, a column temperature of 30°C, and a flow rate of 1.5 mL/min. The effect of column temperature shows that increasing Rt and T further, with T exceeding the acceptable criterion (more than 2.0). The column oven at 30°C and a decrease in the organic modifier content in the mobile phase increased Rt and T beyond acceptable criteria. The effect of flow rate shows that as it increases, Rt and T decrease. However, all conditions indicate that the T exceeds the criteria, as shown in Table 1. TEA was added to the 10 mM phosphate buffer (pH 7.0 ± 0.05) at 0.02% to improve the peak shape of the AZT chromatogram under the optimized HPLC conditions.

The improvement in peak symmetry can be attributed to TEA masking residual silanol groups on the stationary phase. AZT is a basic compound and tends to interact with silanol groups on the silica surface, resulting in peak tailing. The addition of TEA reduces these secondary interactions, leading to a more symmetrical peak shape and improved chromatographic performance.

The optimized chromatogram was obtained using 0.02% TEA:ACN (60:40) ratio, a column temperature of 30°C, and a flow rate of 2.0 mL/min. The chromatogram of the selected condition is shown in Figure 2. Unlike pharmacopoeial procedures, which require distinct chromatographic conditions for each dosage form, the proposed method offers a unified analytical approach. For instance, USP methods for AZT tablets, capsules, and suspensions utilize different column types, mobile phase compositions, and pH conditions. These variations increase analytical complexity and diminish practicality for routine quality control.

A single C18 column and a consistent mobile-phase system were used for all investigated dosage forms, demonstrating that the proposed method simplifies analytical procedures while maintaining satisfactory chromatographic performance.

Compared with previously reported HPLC methods for AZT in pharmaceutical dosage forms, the proposed method uses a simpler mobile-phase composition and a conventional C18 column operated under isocratic conditions. Although several existing methods demonstrate acceptable chromatographic performance, they often require complex analytical conditions, such as complex mobile-phase components.[24,27] Reported methods typically use mobile-phase pH values between 6.5 and 8.0, flow rates of 0.7 to 1.5 mL/min, and column temperatures of 40°C–60°C, resulting in retention times of 3.4–15 min.[21–27]

Although satisfactory peak symmetry, indicated by a *T* less than 2, has been achieved under these conditions,[21–27] some methods require higher buffer concentrations or specific chromatographic modifications to control peak tailing.[21,23,26] These adjustments can increase system complexity, potentially reduce method robustness in routine analytical applications, and shorten column lifetime.

In contrast, the addition of a small amount of TEA in the present study significantly improved peak symmetry and reduced tailing. This modification enabled the use of a simpler

Table 1. Optimization of the HPLC Condition for the Development of AZT Analysis (*n* = 3).

Mobile Phase System	Flow Rate (mL/min)	Temperature (°C)	Rt (min)	*N*	*T*	Analysis Time (min)
Buffer:ACN (60:40, v/v)	1.5	30	7.279 ± 0.04	7851 ± 6.66	2.80 ± 0.53	15
Buffer:ACN (60:40, v/v)	1.5	35	8.915 ± 0.21	5780 ± 24.34	3.28 ± 0.24	15
Buffer:ACN (60:40, v/v)	1.5	40	10.105 ± 0.19	7128 ± 17.06	3.21 ± 0.09	15
Buffer:ACN (65:35, v/v)	1.5	30	8.431 ± 0.04	9065 ± 21.70	3.21 ± 0.13	15
Buffer:ACN (70:30, v/v)	1.5	30	9.188 ± 0.01	8016 ± 22.87	3.35 ± 0.12	15
Buffer:ACN (75:25, v/v)	1.5	30	10.777 ± 0.05	7085 ± 26.35	3.43 ± 0.17	15
Buffer:ACN (80:20, v/v)	1.5	30	11.722 ± 0.02	5936 ± 24.56	3.35 ± 0.78	15
Buffer:ACN (60:40, v/v)	2.0	30	5.734 ± 0.06	6465 ± 22.07	2.22 ± 0.03	10
Buffer with 0.02%TEA:ACN (60:40, v/v)	2.0	30	5.165 ± 0.01	5916 ± 5.20	1.76 ± 0.02	10

Note: Buffer was 10 mM phosphate buffer pH 7.0.

mobile phase system under isocratic conditions, eliminating the need for elevated buffer concentrations or extensive optimization. Such simplification enhances the method's practicality for routine quality control analysis.

This modification enables efficient separation within a shorter analysis time, 10 min. These findings demonstrate that the proposed method achieves comparable chromatographic performance under milder and more practical analytical conditions. Collectively, these characteristics render the proposed method more suitable for routine quality control applications.

Method Validation

Rt, *T*, and *N* were calculated to evaluate the system's applicability.

System Suitability

The optimized RP-HPLC method was evaluated for system suitability by calculating parameters including *Rt*, *N*, *T*, and RSD. All system suitability results were satisfactory and met the established acceptance criteria. The acceptance criteria followed in the general chapter <621> chromatography of USP44-NF39,[36] *N* not less than 1,000, *T* not more than 2.0, and RSD not more than 2.0%. In addition, no peak was detected at the retention time of AZT in any blank injection throughout the analytical sequence, which demonstrates the absence of significant carryover under the proposed chromatographic conditions. The data are shown in Table 2.

Specificity and Selectivity

The ingredients of the formulations in each dosage form and their functions in this study are described in Table 3.

Table 2. System Suitability Parameter of AZT Analysis in Five Replications.

Parameter	Results	Acceptance criteria
Retention time (Rt)	5.171 ± 0.01	–
Theoretical plates (*N*)	6010 ± 26.22	Not less than 1,000
Tailing factor (*T*)	1.80 ± 0.01	Not more than 2.0
RSD	0.92	Not more than 2.0%

Table 3. Typical Excipients and Their Functions in Common Azithromycin Dosage Forms.

Dosage Form	Excipient	Function
Film-coated tablets (white tablet)	Methylcellulose	Diluent/filler; improves compressibility
	Lactose monohydrate	Diluent; enhances tablet bulk
	Corn starch	Disintegrant; facilitates tablet breakup
	Povidone (PVP)	Binder; improves granule cohesion
	Magnesium stearate	Lubricant; reduces friction during compression
	Hypromellose (HPMC)	Film-forming agent
	Titanium dioxide	Opacifier; provides white color
	Polyethylene glycol (PEG) 400/4000	Plasticizer; improves film flexibility
	Glycerol	Plasticizer; improves film flexibility
	Propylene glycol	Plasticizer
	Stearic acid	Lubricant
	Talcum	Glidant/anti-adherent
Capsules	Sodium starch glycolate	Disintegrant; promotes capsule content dispersion
	Magnesium stearate	Lubricant
	Stearic acid	Lubricant
	Talcum	Glidant
Dry syrup/dry suspension	Sucrose	Sweetener; improves palatability
	Sorbitol	Sweetener; improves palatability
	Xanthan gum	Suspending agent; stabilizes dispersion
	Acacia	Suspending agent; stabilizes dispersion
	Citric acid/sodium citrate	Buffering agent; controls pH
	Sodium benzoate	Preservative
	Methyl paraben/propyl paraben	Preservative
	Orange flavor	Improves taste
	Tartrazine	Provides visual appearance
	Ascorbic acid	Antioxidant
	Propylene glycol	Co-solvent/wetting agent
	Mannitol	Sweetener and bulking agent; improves palatability
	Span 80	Surfactant and wetting agent; improves particle dispersion
	Tween 80	Surfactant and wetting agent; improves particle dispersion

The specificity results demonstrated that common excipients in the investigated dosage forms did not interfere with AZT determination under the optimized chromatographic conditions. In film-coated tablets and capsules, excipients such as lactose monohydrate, mannitol, starch derivatives, magnesium stearate, talc, HPMC, PEG, and titanium dioxide primarily function as diluents, disintegrants, lubricants, glidants, or coating agents. These substances are either poorly retained, insoluble and removed during sample filtration, or do not exhibit sufficient UV response at the retention time of AZT. Consequently, no co-eluting peaks were observed in the placebo chromatograms.

In dry syrup and dry suspension formulations, excipients such as sucrose, sorbitol, saccharin sodium, sodium benzoate, sodium citrate, xanthan gum, acacia, Tween 80, and flavoring agents serve as sweeteners, preservatives, buffering agents, suspending agents, wetting agents, or palatability enhancers. Although certain ionic compounds and surfactants could potentially influence chromatographic behavior, their effects were minimized by the optimized mobile phase containing TEA, which suppresses secondary silanol interactions on the C18 stationary phase. Additionally, polymeric or high-molecular-weight excipients, including xanthan gum, methylcellulose, HPMC, and acacia, were not expected to elute as sharp UV-absorbing peaks under the current conditions and were largely removed or reduced during sample preparation and filtration.

These results confirm that the developed method provides sufficient selectivity for AZT in various formulation matrices, including both solid and reconstituted liquid dosage forms. No peaks from blank or placebo matrices were detected at the retention time of AZT, demonstrating that the method is selective for AZT determination in all tested dosage forms, as shown in Figure 3.

Among the dosage forms evaluated, the dry suspension formulation demonstrated the most complex excipient profile, with multiple excipient-related peaks detected before the AZT peak (Figure 5C and 5D). Consequently, additional peak purity analysis using a photodiode array (PDA) was conducted to confirm peak specificity in this matrix. The UV spectra obtained at the leading edge (4.96 min), apex (5.04 min), and trailing edge (5.11 min) of the AZT peak were highly superimposable, with spectral similarity values ranging from 999.99 to 1000.00. These findings indicate excellent spectral homogeneity of the AZT peak and support the absence of co-eluting excipient components, thereby confirming the specificity of the proposed method for the dry suspension formulation.

Linearity

The calibration curve demonstrated good linearity over the investigated concentration range (0.80–1.20 mg/mL), with a correlation coefficient (R^2) of 0.9985. The %residuals were within ±2% and randomly distributed around zero, indicating the adequacy of the linear regression model and the absence of significant systematic deviation. The data are shown in Figure 4 and Table 4.

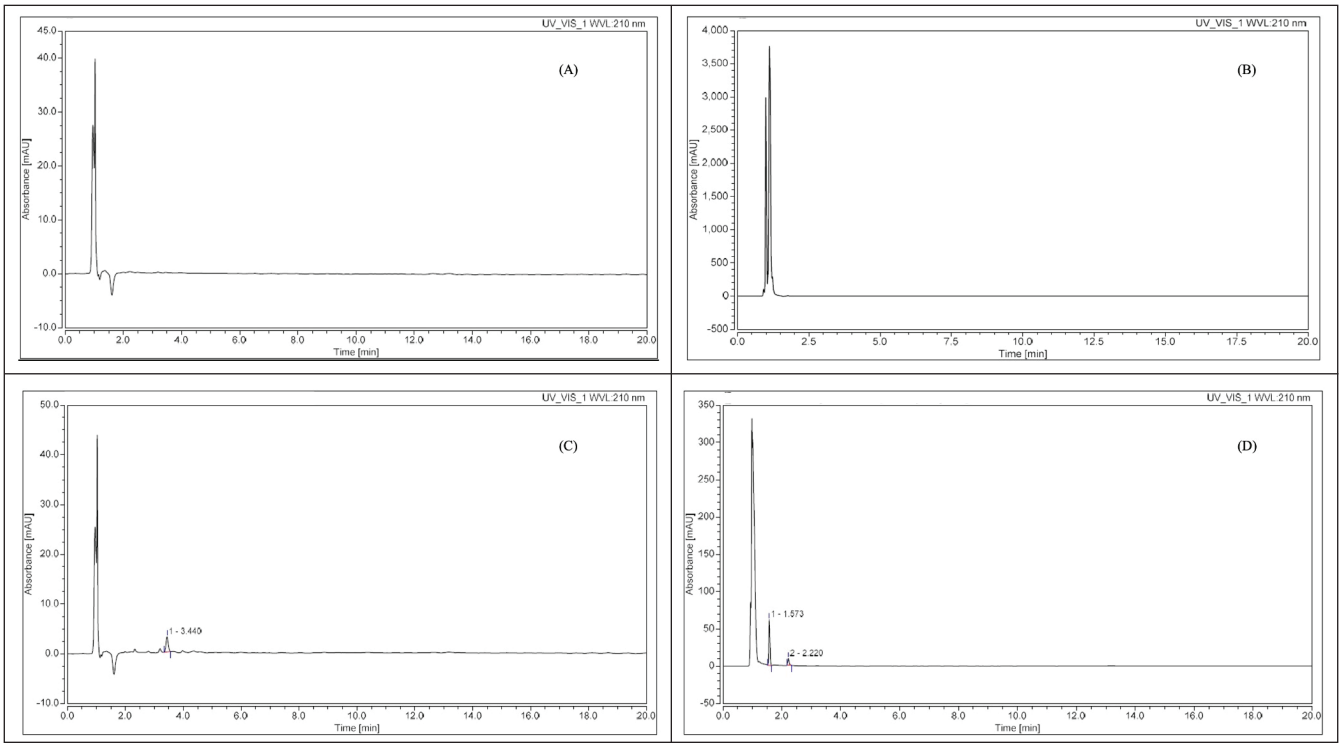

Figure 3. Chromatogram of (A) Blank Interference, (B) Excipients of Capsule, (C) Excipients of Core and Film-coated Tablets, and (D) Excipients of Dry Syrup and Dry Suspension.

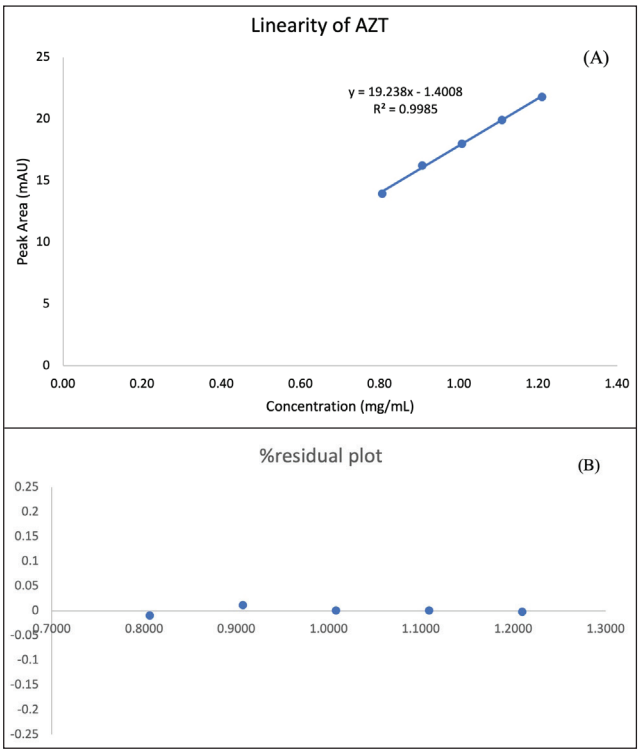

Figure 4. Linearity of AZT (A) Calibration Curve Constructed by Plotting the Peak Area Versus Concentration. (B) %Residual Plot for the Calibration Curve.

Accuracy

In accordance with AOAC guidelines[35] and the 0.01% analyte recommendation, the percentage recovery of AZT remained within the acceptable range of 90%–107%. The developed method exhibited both precision and accuracy, as shown in Table 4.

The accuracy of the proposed method was evaluated at three concentration levels (80%, 100%, and 120%) with six replicates at each level. The percentage recoveries ranged from 100.41% to 101.00%, with %RSD values below 1%. The 95% confidence intervals were narrow, indicating good reliability and accuracy of the method in accordance with ICH Q2(R2).[34]

Precision

Precision of the proposed method was assessed by evaluating repeatability and intermediate precision, using six replicate determinations at each concentration level. The resulting %RSD values ranged from 0.55% to 0.91% (Table 4), which are substantially lower than the AOAC acceptance criterion of 5.3%. These findings indicate that the proposed method exhibits excellent precision and reproducibility.

LOD and LOQ

The calculated LOD and LOQ values were 0.0232 and 0.0703 mg/mL, respectively. These values indicate adequate sensitivity for the intended assay application.

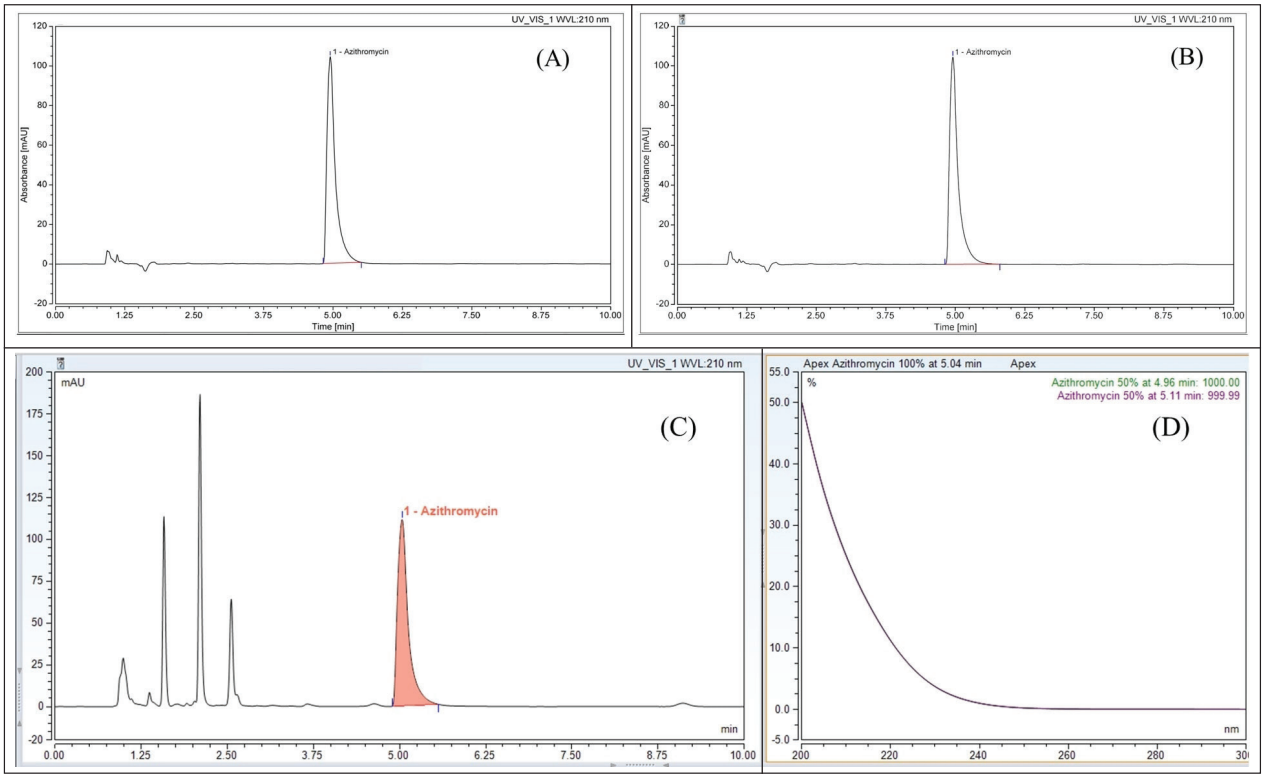

Figure 5. Representative Chromatograms of AZT in (A) Film-coated Tablet, (B) Capsule, and (C) Dry Suspension Formulations. Due to the Increased Complexity of Excipients in the Dry Suspension Matrix, Peak Purity Assessment Using Photodiode Array (PDA) Detection Was Conducted for the AZT Peak. (D) PDA Spectral Overlay Obtained Across the AZT Peak in the Dry Suspension Sample Demonstrates Spectral Homogeneity and Confirms Peak Specificity.

Table 4. Validation Data of the Developed HPLC Method of AZT.

Linearity					
Linearity Range		0.80–1.20 mg/mL			
Coefficient of Determination (R^2)		0.9985			
Slope		19.238			
y-Intercept		−1.4008			
LOD		0.0232 mg/mL			
LOQ		0.0703 mg/mL			
Accuracy (n = 6)					
Concentration (mg/mL)	%Recovery ± SD			95%CI	
0.80	101.00 ± 0.72			100.24–101.74	
1.00	100.61 ± 0.85			99.73–101.47	
1.20	100.41 ± 0.71			99.69–101.13	
Precision (n = 6)					
	Repeatability			Intermediate Precision	
Concentration (mg/mL)	%Recovery ± SD	%RSD	Concentration (mg/mL)	%Recovery ± SD	%RSD
0.80	101.00 ± 0.72	0.71	0.80	100.11 ± 0.91	0.91
1.00	100.61 ± 0.85	0.85	1.00	100.84 ± 0.92	0.91
1.20	100.41 ± 0.71	0.71	1.20	99.82 ± 0.55	0.55
Robustness (n = 3)					
Parameter	Level	Rt ± SD (min)	Peak Area ± SD	N ± SD	T ± SD
Normal condition[a]	–	5.166 ± 0.01	17.437 ± 0.13	5969.40 ± 50.26	1.78 ± 0.03
Detection wavelength (nm)	209	5.166 ± 0.01	18.767 ± 0.07	5967.20 ± 49.63	1.78 ± 0.03
	211	5.166 ± 0.01	16.263 ± 0.14	5970.60 ± 55.04	1.78 ± 0.03
Flow rate (mL/min)	1.90	5.315 ± 0.01	18.063 ± 0.05	5803.40 ± 14.93	1.80 ± 0.01
	2.10	5.004 ± 0.01	17.167 ± 0.08	5864.60 ± 82.43	1.68 ± 0.02
pH	6.8	4.450 ± 0.01	16.201 ± 0.01	6049.00 ± 36.49	1.62 ± 0.02
	7.2	7.193 ± 0.01	21.205 ± 0.07	5475.20 ± 48.85	1.88 ± 0.03
Column temperature (°C)	29	5.023 ± 0.01	17.361 ± 0.07	5995.60 ± 42.81	1.75 ± 0.03
	31	5.253 ± 0.01	17.476 ± 0.09	5982.40 ± 36.13	1.82 ± 0.04

Note: [a]Nominal conditions, pH of buffer 7.0, column temperature 30°C, flow rate 2.0 mL/min.

Robustness

The robustness of the proposed method was assessed by introducing controlled variations in buffer pH, column temperature, flow rate, and detection wavelength. For column temperature, flow rate, and detection wavelength, only minor changes in chromatographic parameters were observed, with no significant effects on *Rt*, peak area, *N*, or *T*.

In contrast, variation in buffer pH produced a noticeable change in *Rt*, demonstrating the method's sensitivity to pH. Despite this, system suitability parameters, such as *N* and *T*, remained within acceptable limits, thereby confirming the method's robustness under controlled conditions. The corresponding data are presented in Table 4.

While all robustness parameters met the predefined acceptance criteria, changes in the mobile phase pH resulted in significant alterations in both retention time and peak area. This outcome indicates that the chromatographic behavior of AZT is strongly influenced by its ionization state. In contrast, intentional modifications to column temperature, flow rate, and detection wavelength had only minor effects on chromatographic performance.

These results demonstrate that the proposed method is suitable for routine analysis if the mobile phase pH is strictly controlled. It is therefore recommended to maintain the buffer pH within ±0.05 units of the optimized value (pH 7.0) to ensure consistent chromatographic performance and reproducibility.

In-use Standard Solution Stability

The in-use stability of the AZT standard solution was assessed at room temperature over 24 h. The percentage of remaining analyte ranged from 99.91% to 100.65% relative to the initial response at 0 h. The percentage change remained within ±1%, demonstrating that the standard solution was stable under the evaluated conditions. The results are shown in Table 5.

Table 5. In-use Stability of AZT Standard Solution (n = 5) at Room Temperature. Results are Expressed as Mean ± SD.

Time (h)	Peak Area	%Remaining	%Change
0	18.063 ± 0.03	100.00	–
12	18.181 ± 0.05	100.65	0.65
24	18.047 ± 0.05	99.91	–0.09

Note: Remaining was calculated relative to the initial response at 0 h.

The stability of sample solutions was not formally assessed in this study and, as such, cannot be conclusively determined. All sample solutions were prepared and analyzed within 6 h to minimize potential alterations due to storage. Since reconstituted dry suspension formulations contain preservatives, buffering agents, and other excipients that may affect AZT stability during prolonged storage, freshly prepared sample solutions are advised. Additional research evaluating the stability of the sample solution under various storage conditions would provide important support for the long-term routine application of this method.

Comparison with Previously Reported HPLC Analysis Methods for AZT

Compared with previously reported HPLC methods for AZT determination, the proposed method offers several analytical advantages, including chromatographic simplicity, improved peak symmetry, and suitability for routine quality control analysis. As summarized in Table 6, previously reported HPLC methods for AZT determination employ a variety of chromatographic strategies depending on the analytical purpose. These methods differ in stationary phases, mobile phase compositions, buffer systems, column temperatures, elution modes, and detector configurations that have been optimized to achieve satisfactory chromatographic performance and analytical reliability. These chromatographic strategies are generally intended to improve chromatographic performance, including suppression of secondary interactions between protonated AZT and residual silanol groups on silica-based stationary phases.

This study achieved satisfactory chromatographic performance using a conventional silica-based C18 column under mild isocratic conditions. Incorporation of TEA into the mobile phase minimized silanol-related secondary interactions and improved peak symmetry without the need for highly optimized stationary phases or aggressive chromatographic conditions. The proposed method also provided a short retention time and analytical runtime while maintaining acceptable chromatographic efficiency and robustness. Unlike several previously reported methods that focused on a specific dosage form, the developed method was successfully applied to multiple pharmaceutical formulations, including tablets, capsules, dry syrup, and dry suspension. These results indicate that the proposed method offers a practical and simplified alternative for routine AZT analysis in pharmaceutical quality control laboratories.

Application of the Developed Method

The validated HPLC method was utilized to analyze selected commercial AZT products available in Thailand, including film-coated tablets, capsules, dry syrup, and dry suspension. Assay results ranged from 99.42% to 103.65% of the label claim, with RSD values below 2.0%. The slight variation observed among different formulations may be attributed to differences in formulation composition and excipients, particularly in liquid dosage forms such as dry suspensions. Excipients, including suspending agents, sugars, and viscosity modifiers, may influence drug extraction efficiency and chromatographic response in assay results. However, the excipient of the formulation did not interfere with the AZT chromatogram, as shown in Figure 5.

The sonication step was implemented to achieve complete extraction of AZT from pharmaceutical formulations, rather than to optimize extraction kinetics. Given the relatively low aqueous solubility of AZT, a 30 min sonication time was chosen as a practical condition to ensure consistent sample preparation across all dosage forms evaluated in this study.

All tested products met the pharmacopeial acceptance limits specified in the corresponding USP and BP monographs for their respective dosage forms. The corresponding data are presented in Table 7.

The developed method was evaluated against the percentage of label claims for Zithromax® tablets. No statistically significant difference was observed between the proposed method and the USP method[28] for AZT tablet assay using Welch's t-test ($t = -0.28$, df = 3, $p = .80$), indicating that the assay results obtained by the two methods were comparable under the conditions evaluated. Although the comparison was based on a limited number of replicate determinations (n = 3 per method), the results support the suitability of the proposed method for routine assay of AZT tablets. The corresponding data are presented in Table 8.

The chromatographic strategy utilized in this study may be extended to other basic pharmaceutical compounds that demonstrate secondary interactions with residual silanol groups on silica-based C18 stationary phases. Analytical HPLC methods employing amine-containing mobile phase modifiers have been widely reported for basic pharmaceutical compounds. Examples include atorvastatin, methotrexate, leflunomide, erythromycin, ciprofloxacin, norfloxacin, levofloxacin, and related fluoroquinolones, where triethanolamine was used as the mobile phase modifier to improve chromatographic performance.[13,14,37–40] The present method employs triethylamine as the mobile phase modifier. The beneficial effect of these amine modifiers is generally attributed to suppression of secondary interactions between protonated basic analytes and residual silanol groups on silica-based stationary phases, thereby improving peak symmetry and chromatographic efficiency.[41–43]

Table 6. Comparison of the Proposed HPLC Method with Previously Reported Methods for AZT Determination.

Reference	Column	Mobile Phase	Flow Rate (mL/min)	Injection Volume (µL)	Detection Wavelength (nm)	Temperature (°C)	RT (min)	*T*	Analysis Time (min)	Dosage Form
21	C18	38.5 mM phosphate buffer pH 8.0:ACN (40:60)	1.2	20	210	40	3.40	–	–	Standard AZT and AZT tablets
22	C18	20 mM phosphate buffer pH 8.0:MeOH (10:90)	1.5	500	210	Room temperature	7.23	–	10	Standard AZT
23	C18	0.05 M ammonium acetate pH 8.0:ACN (60:40)	0.8	10	ELSD detector	40	16.0	0.68–1.94	25	Standard AZT, raw material, capsules, injection, and tablets
24	C18	Methanol, ACN and 200 mM phosphate buffer pH 8.0 (60:30:10)	1.0	20	212	25	4.80	–	15	Oral suspension
25	C18	Methanol: 200 mM phosphate buffer pH 8.0 (90:10)	1.2	50	210	40	5.80	1.02	11	Capsule, dry suspension, and pow-der for solution for infusion
26	C18	30 mM ammonium acetate solution pH = 6.8:ACN (18:82, v/v)	0.7	10	210	60	7.95	1.50	20	Bulk, and self-emulsifying drug delivery system
27	C18	ACN:100 mM KH_2PO_4 pH 6.5:100 mM tetra butyl ammonium hydroxide pH 6.5:water (25:15:1:59 v/v)	1.0	10	215	43	8.10	–	30	Tablet and dry suspension
Proposed method	C18	10 mM phosphate buffer with 0.02% TEA:ACN (60:40)	2.0	40	210	30	5.17	1.78	10	Tablet, capsule, dry syrup, and dry suspension

Table 7. Application of the Developed HPLC Method for the Analysis of Selected Commercial Pharmaceutical Products Available in Thailand (n = 3).

Dosage Form	Product	Label (mg/Unit)	%Label Claim	%RSD
Tablet	Zithromax®	250 mg/tablet	99.71	0.44
Capsule	Cap-A	250 mg/capsule	101.07	0.46
	Cap-B	250 mg/capsule	101.07	0.79
	Cap-C	250 mg/capsule	101.04	0.77
Dry syrup	DS-A	200 mg/5 mL	101.13	1.21
Dry suspension	Zithromax®	200 mg/5 mL	103.65	0.12
	DSu-A	200 mg/5 mL	99.42	0.66
	DSu-B	200 mg/5 mL	102.20	0.66

Note: Product codes (Cap-A, DS-A, DSu-A, and DSu-B) represent locally manufactured products in Thailand. Zithromax® is the reference product.

Table 8. Statistical Comparison Between the Developed Method and the USP44-NF39 Method for Azithromycin Tablet Assay (n = 3).

Method	%Label Claim (Mean ± SD, n = 3)	%RSD	Mean Difference (USP-proposed Method)	95%CI of the Difference	t Value	df	p Value
Proposed method	99.71 ± 0.44	0.44	−0.08	−0.99 to 0.83	−0.28	3	.80
USP method	99.63 ± 0.23	0.23					

Note: Statistical comparison between the proposed method and the USP method was performed using Welch's t-test. No formal equivalence margin was pre-specified; therefore, the results are interpreted as showing no statistically significant difference rather than formal equivalence.

Conclusion

A straightforward RP-HPLC-UV method was developed and validated for the quantification of AZT in various pharmaceutical dosage forms. The method uses a conventional C18 column and an isocratic mobile phase of 10 mM phosphate buffer (pH 7.0 ± 0.05) containing 0.02% TEA and ACN. Validation demonstrated satisfactory linearity, accuracy, precision, robustness under controlled chromatographic conditions, and in-use standard solution stability, meeting the requirements of ICH Q2(R2) guidelines. Application to commercial products, including tablets, capsules, dry syrup, and dry suspension, confirmed compliance with pharmacopoeial criteria. This method provides a simplified, unified approach suitable for routine quality control of AZT across multiple dosage forms using widely accessible chromatographic conditions.

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Authors' Contribution

PS contributed to the conception and design of the study, data acquisition, data analysis and interpretation, statistical analysis, critical revision of the manuscript, supervision, and final approval of the manuscript. NP, PC, and BS contributed to the study's conception and design, data acquisition, manuscript drafting, administrative, technical, or material support, and final approval of the manuscript. AP contributed to administrative, technical, or material support and final approval of the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of Conflicting Interests

The AZT working standard utilized in this study was provided by Siam Pharmaceutical Co., Ltd. Siam Pharmaceutical Co., Ltd., did not participate in the study design, data collection, data analysis, interpretation of results, manuscript preparation, or the decision to publish. Additionally, none of the commercial pharmaceutical products assessed in this study were produced by Siam Pharmaceutical

Co., Ltd. The authors declare no competing financial or personal interests that could have influenced the research presented in this article.

Ethical Approval

This study does not involve human participants, human data, or animal subjects. Therefore, ethical approval and informed consent were not required.

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Informed Consent

Not applicable.

Use of Artificial Intelligence-assisted Tools

The authors declare that no artificial intelligence (AI) tools were used to generate, interpret, or write the scientific content of this manuscript. AI-assisted tools, specifically ChatGPT and Grammarly, were used solely for minor language editing, including improvements in grammar, spelling, clarity, and readability. These tools were not used to generate, modify, analyze, or enhance any scientific figures, chromatograms, tables, experimental data, results, or conclusions presented in this study. The authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

Supplemental Material

Supplemental material for this article is available online.

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Abbreviations

ACN = Acetonitrile

AZT = Azithromycin

BP = British Pharmacopoeia

df = Degrees of freedom

HPMC = Hydroxypropyl methylcellulose

LOD = Limit of detection

LOQ = Limit of quantitation

N = Theoretical plate

PEG = Polyethylene glycol

PVP = Polyvinylpyrrolidone

RP-HPLC = Reversed-phase high-performance liquid chromatography

Rt = Retention time

RSD = Relative standard deviation

T = Tailing factor

TEA = Triethylamine

USP = United States Pharmacopoeia

UV = Ultraviolet