

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR THE QUANTIFICATION OF IMPURITY A IN DILTIAZEM TABLETS

Nemanja Turković^{1,2}, Milena Turković³, Željka Bešović², Branka Ivković⁴

¹University of Montenegro, Faculty of Medicine, Study Program Pharmacy, Podgorica, Montenegro

²Institute for Medicines and Medical Devices of Montenegro, Podgorica, Montenegro

³The Health Institution of the Pharmacy of Montenegro "Montefarm", Podgorica, Montenegro

⁴University of Belgrade, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Belgrade, Serbia

Contact: Nemanja Turković

Kruševac bb, Podgorica, Montenegro

Email: nemanja.t@ucg.ac.me

A simple and precise RP-HPLC method was developed and validated for the quantification of impurity A in diltiazem pharmaceutical dosage forms. Chromatographic analysis was performed using a Zorbax Eclipse XDB-C18 (150 x 4.6 mm) 5 µm column under isocratic elution conditions with a mobile phase consisting of phosphate buffer (pH 3.4) and acetonitrile in a ratio of 70 : 30 (v/v), at a flow rate of 1.2 mL/min and a column temperature of 25 °C. Detection was carried out at 210 nm. The developed method was validated in accordance with ICH guidelines. The following analytical parameters were evaluated during validation: specificity, linearity, precision, accuracy, robustness, the limit of detection (LOD) and the limit of quantification (LOQ) for the investigated impurity. The method demonstrated linearity over the concentration range of 98–980 ng/mL ($r = 0.99992$) for the impurity A. Good precision (Relative Standard Deviation (RSD) < 3%), accuracy (recovery in the range of 98.70–100.14%), and robustness were confirmed. LOD and LOQ were 18.52 ng/mL and 56.13 ng/mL, respectively. The method reliably quantified impurity A in diltiazem tablets, meeting both USP monograph and manufacturer specification limits.

Key words: diltiazem; impurity A; method validation; HPLC

**RAZVOJ I VALIDACIJA RP-HPLC METODE ZA ODREĐIVANJE NEČISTOĆE A U
TABLETAMA DILTIAZEMA**

Nemanja Turković^{1,2*}, Milena Turković³, Željka Bešović², Branka Ivković⁴

¹Univerzitet Crne Gore, Medicinski fakultet, Studijski program Farmacija, Podgorica, Crna Gora

²Institut za lijekove i medicinska sredstva, Podgorica, Crna Gora

³Zdravstvena ustanova Apoteke Crne Gore "Montefarm", Podgorica, Crna Gora

⁴Univerzitet u Beograd, Farmaceutski fakultet, Katedra za farmaceutsku hemiju, Beograd, Srbija

Kontakt: Nemanja Turković

Kruševac bb, Podgorica, Crna Gora

E-mail: nemanja.t@ucg.ac

U ovom radu prikazan je razvoj i validacija jednostavne i precizne RP-HPLC metode za određivanje sadržaja nečistoće A diltiazema u doziranim farmaceutskim oblicima. Hromatografska analiza je izvedena korišćenjem Zorbax Eclipse XDB-C18 (150 x 4,6 mm) 5 µm kolone pod izokratskim uslovima eluiranja sa mobilnom fazom fosfatni pufer pH 3,4 i acetonitril u odnosu 70 : 30 (v/v), brzinom protoka od 1,2 mL/min i radnom temperaturom od 25 °C. Detekcija je vršena na talasnoj dužini 210 nm. Razvijena metoda validirana je prema ICH smernicama. Tokom validacije testirani su sledeći analitički parametri: specifičnost, linearnost, preciznost, tačnost, robusnost kao i limit detekcije (LOD) i limit kvantifikacije (LOQ) za ispitivanu nečistoću. Metoda je linearna u opsegu koncentracija od 98–980 ng/mL ($r = 0.99992$) za ispitivanu nečistoću. Dokazana je dobra preciznost (Relativna standardna devijacija (RSD) < 3%), tačnost (Recovery u opsegu 98,70–100,14%) i robusnost metode. LOD je bio 18,52 ng/mL, dok je LOQ bio 56,13 ng/mL. Metoda je pouzdano odredila sadržaj nečistoće A u tabletama diltiazema, ispunjavajući zahteve kako USP monografije, tako i specifikacije proizvođača.

Ključne reči: diltiazem; nečistoća A; validacija analitičke metode; HPLC

Introduction

Diltiazem (Figure 1) is a benzothiazepine derivative and a calcium channel blocker. It inhibits the influx of extracellular calcium through slow channels located in the cell membranes of smooth muscle cells (cardiac and vascular). Diltiazem is indicated in adults for: chronic stable angina pectoris; prevention of anginal attacks; vasospastic angina (Prinzmetal's angina); essential hypertension (1).

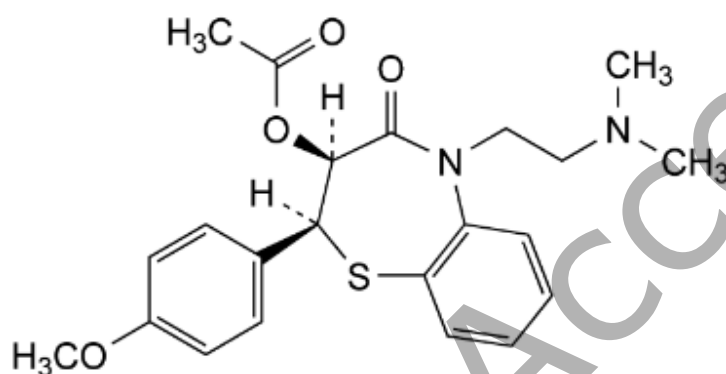


Figure 1. Chemical structure of diltiazem

Chemically, diltiazem is (2S,3S)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate. It is a basic molecule because it contains a tertiary aliphatic amino group (pKa 8.06) (2). This structural feature renders it unstable in the presence of light, moisture, and atmospheric oxygen; therefore, it is used therapeutically in the form of its hydrochloride salt. In addition to the tertiary aliphatic amino group, diltiazem also contains an ester functional group, which further contributes to its instability. It is a lipophilic molecule with a logP value of 2.8 (2). Diltiazem contains two chiral centers at positions 2 and 3, allowing for four possible stereoisomers; however, only the (2S,3S), i.e., the *cis*-isomer, exhibits the desired pharmacological activity (3).

Impurity A ((+)-*trans*-diltiazem, (2*R*-*trans*)-diltiazem) (Figure 2) is the *trans*-isomer of diltiazem. Its formation is mainly associated with the stereochemical characteristics of diltiazem synthesis, where insufficient stereoselectivity may lead to the presence of the *trans*-isomer as a process-related impurity. In addition, the existence of two chiral centers in the diltiazem structure allows for potential *cis*–*trans* isomeric variations under certain conditions during manufacturing and/or storage of the finished product.

The presence of impurity A is relevant from a quality control perspective, since it may influence the accurate quantification of diltiazem and contribute to the overall related substances profile. Consequently, its determination is essential for ensuring the quality and regulatory compliance of pharmaceutical products containing diltiazem. Both the European Pharmacopoeia and USP specify impurity A as a controlled impurity with defined limits, highlighting the importance of its reliable monitoring in routine analysis of finished dosage forms (4).

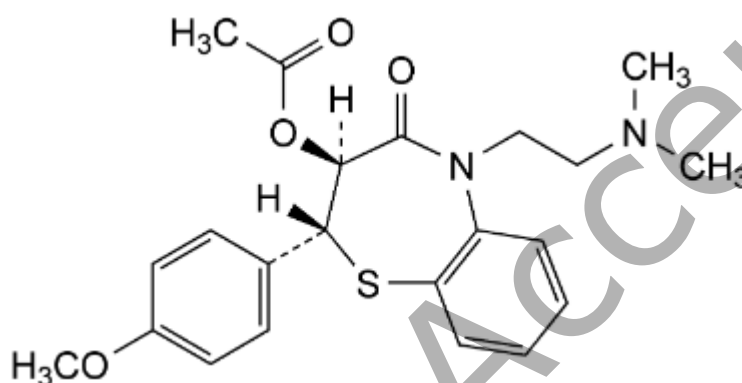


Figure 2. Chemical structure of impurity A of diltiazem

A detailed literature survey indicates that only a few analytical methods have been published for the determination of related substances in diltiazem pharmaceutical dosage forms (4, 5). Previously reported chromatographic methods for the analysis of diltiazem-related substances have mainly focused on the determination of degradation products, process-related impurities, and related compounds in bulk drug substances and pharmaceutical formulations. Quaglia et al. reported an HPLC and LC-MS approach for the analysis of diltiazem and its related substances, including the separation of stereoisomeric compounds. Chatpalliwar et al. developed a validated gradient HPLC method for the determination of diltiazem hydrochloride and related substances in bulk drug and tablet formulations. However, limited information is available regarding simple and specifically validated RP-HPLC methods intended for the quantitative determination of impurity A in finished diltiazem tablets. Therefore, the

development of a selective and validated RP-HPLC method for impurity A determination remains relevant for routine quality control of pharmaceutical dosage forms.

The aim of this research was development and validation of the appropriate reversed-phase HPLC (RP-HPLC) method for the quantification of impurity A in diltiazem pharmaceutical dosage forms.

Material and methods

Equipment

The following equipment was used: liquid chromatograph: HP 1100, Hewlett Packard (detector: UV/VIS; binary pump: HP 1100, model G 1312 A; injector: Rheodyne; column thermostat: HP 1100, model G 1316 A; data processing: ChemStation and Origin 7), analytical balance: Sartorius BP 1215, pH meter with combined glass electrode: MeterLab PHM220, Radiometer, ultrasonic bath: Sineks Laboratory N° 25860 Mod UCI-75, filtration system: Whatman 47 mm Glass-fiber, membrane filter holder: 0.45 µm Whatman international Ltd Maid Stone, England, microsyringe: Hamilton, Australia, volume 100 µL, membrane sample filters: Machrey-Nagel GmbH&Co Kg.Germany 640/125 mm.

Chemicals and reagents

Diltiazem impurity A CRS was obtained from the European Directorate for the Quality of Medicines & HealthCare (EDQM), Strasbourg, France. Acetonitrile of HPLC grade was supplied by Fischer Scientific, Germany. Orthophosphoric acid, potassium dihydrogen phosphate and triethylamine (all pro analysis grade) were supplied by Merck, Germany. Purified water was produced using a TKA GenPure water purification system from Thermo Electron LED GmbH, Germany. Diltiazem tablets, strength 90 mg were used.

Chromatographic conditions

Based on preliminary experiments evaluating different mobile phase compositions and buffer pH values, the chromatographic conditions selected for the quantification of impurity A were as follows: chromatographic column: Zorbax Eclipse XDB-C18 150 x 4.6 mm, 5µm; mobile phase: phosphate buffer (pH 3.3) and acetonitrile in a volume ratio of 70:30; flow rate: 1.2 mL/min; injected volume: 20 µL; column temperature: 25°C; UV detection: 210 nm. The detection wavelength of 210 nm was selected based on the UV absorption characteristics of

diltiazem and impurity A, providing adequate sensitivity for the determination of impurity A at trace levels while maintaining suitable selectivity of the chromatographic analysis.

Preparation of solutions

The placebo solution was prepared by transferring an accurately weighed amount of placebo mixture equivalent to the average tablet mass (~230 mg) into a 100 mL volumetric flask, dissolving it in solvent with sonication (15 min), diluting to volume, and filtering. An aliquot of the filtrate was further diluted (1:10, v/v) with the same solvent.

A stock solution of impurity A CRS (100 µg/mL) was prepared in acetonitrile and further diluted to obtain an intermediate solution (10 µg/mL). Working solutions for linearity were prepared by appropriate volumetric dilutions to yield concentrations in the range of 98–980 ng/mL and were injected in triplicate.

For repeatability studies, a solution containing 490 ng/mL of impurity A was prepared from the stock solution, while additional dilutions covering low, medium, and high concentration levels were used for intra-day and intermediate precision assessment.

Accuracy was evaluated using placebo matrices spiked with the standard at 80%, 100%, and 120% of the target concentration. Spiked samples and corresponding reference solutions were prepared by volumetric dilution and analyzed in triplicate.

For quantification of impurity A in tablets, a working reference solution (490 ng/mL) was prepared from the stock solution. Sample solutions were obtained by accurately weighing and powdering 20 tablets, transferring an amount equivalent to the average tablet mass into a 100 mL volumetric flask, extracting with solvent under sonication (25 min), diluting to volume, and filtering to obtain a final solution of approximately 900 µg/mL.

Results and discussion

During method development, preliminary experiments were performed to establish suitable chromatographic conditions for the separation of impurity A from diltiazem. Different mobile phase compositions and buffer pH values were evaluated to achieve adequate retention, peak symmetry, and selectivity. The proportion of organic modifier was adjusted to achieve satisfactory resolution within an acceptable analysis time. The selected chromatographic conditions, consisting of phosphate buffer (pH 3.3) and acetonitrile in a 70:30 (v/v) ratio, provided efficient separation of impurity A and diltiazem with suitable chromatographic parameters and were therefore selected for further method validation.

Impurity A exhibits physicochemical properties that differ only slightly from those of diltiazem as the *cis*-isomer. The different spatial orientation of the *p*-methylphenyl group at position 2 of the thiazepine ring in these two isomers enables their chromatographic separation under the selected RP-HPLC conditions, employing a modified octadecylsilyl silica gel as the stationary phase and a mixture of buffer and acetonitrile as the mobile phase. The phosphate buffer, adjusted to pH 3.4, maintained both isomers in their ionized forms throughout the analysis.

System suitability

Prior to chromatographic analysis, the suitability of the RP-HPLC system configured under the specified chromatographic conditions was evaluated. System suitability was verified by repeated injections of the standard solution at the working concentration until consistent peak areas and retention parameters were obtained for three consecutive injections. The obtained system suitability results indicate that the chromatographic system was stable and adequate for analysis. Repeated injections of the standard solution showed consistent peak areas and retention times, confirming system reproducibility. The evaluated parameters (including retention time, capacity factor, and symmetry factor) were within acceptable limits, demonstrating suitability of the chromatographic system prior to sample analysis. The chromatographic parameters for Impurity A are presented in Table 1.

Table 1. Chromatographic parameters for the HPLC analysis of impurity A of diltiazem

Chromatographic parameters	Impurity A
Retention time, t_r	4.38
Retention time, t_o	1.58
Capacity Factor, k'	1.77
Symmetry Factor, A_s	0.90
Resolution, R_s	2.40
Number of theoretical plates, N	6480

Selectivity

Method selectivity was evaluated by injecting, under the specified chromatographic conditions, the placebo solution, the impurity A standard solution, the diltiazem standard solution, and the mixture of diltiazem and impurity A standards, followed by comparison of the retention times of impurity A and diltiazem peaks with the peaks observed in the chromatograms of the placebo and laboratory mixture solutions (Figure 3).

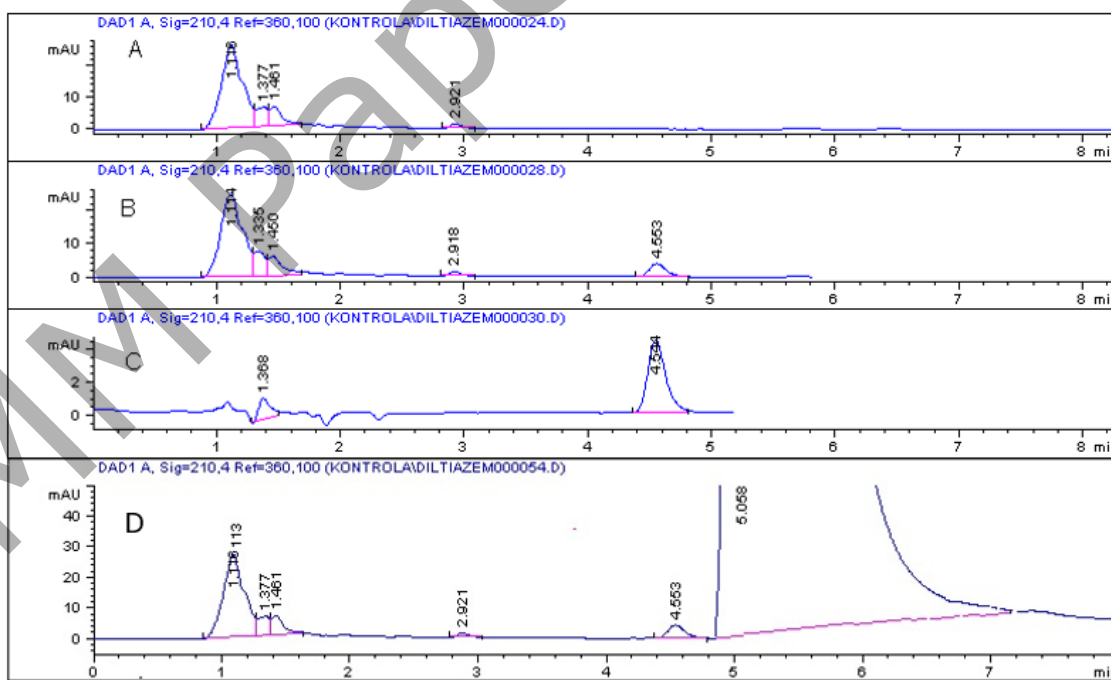


Figure 3. A) Chromatogram of the placebo mixture; B) Chromatogram of the laboratory mixture; C) Chromatogram of impurity A standard D) Chromatogram of diltiazem standard and impurity A standard mixture

In the chromatogram of the placebo mixture solution, no peaks corresponding to the retention time of impurity A were observed, confirming the absence of interference from excipients. The chromatogram of the mixture of diltiazem and impurity A standards (Figure 3D) demonstrated complete separation of the two analytes under the selected chromatographic conditions. The obtained resolution between diltiazem and impurity A was satisfactory ($R_s > 1.5$), confirming adequate chromatographic selectivity and suitability of the proposed method for the determination of impurity A in diltiazem tablets.

Linearity

The linearity of the method was assessed by constructing a calibration curve using the least square method. The calibration curve represents the dependence of the peak area of the standards on the concentration of the standard solutions. The results obtained for impurity A are shown in Figures 4 and 5.

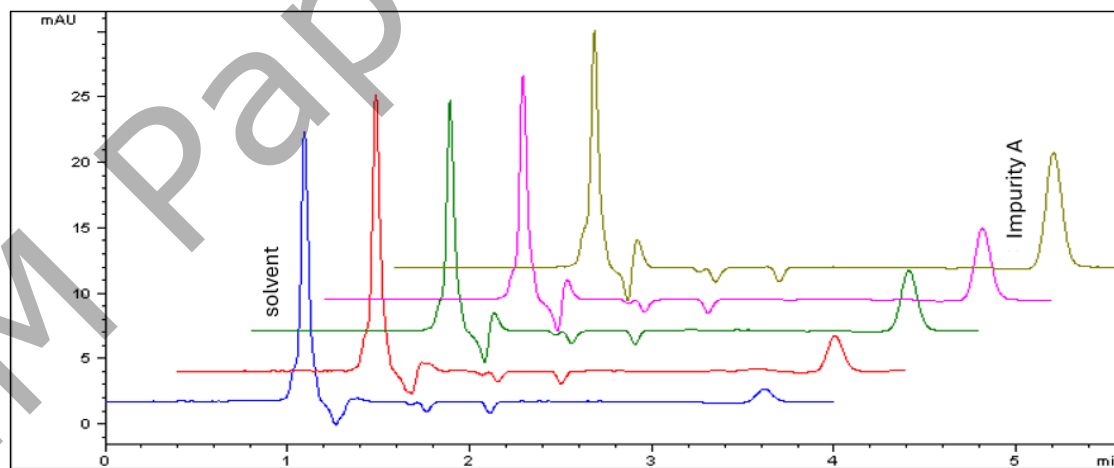


Figure 4. Chromatograms of standard solutions of impurity A at various concentrations, used for the construction of the calibration curve (concentration range from 98 ng/mL to 980 ng/mL)

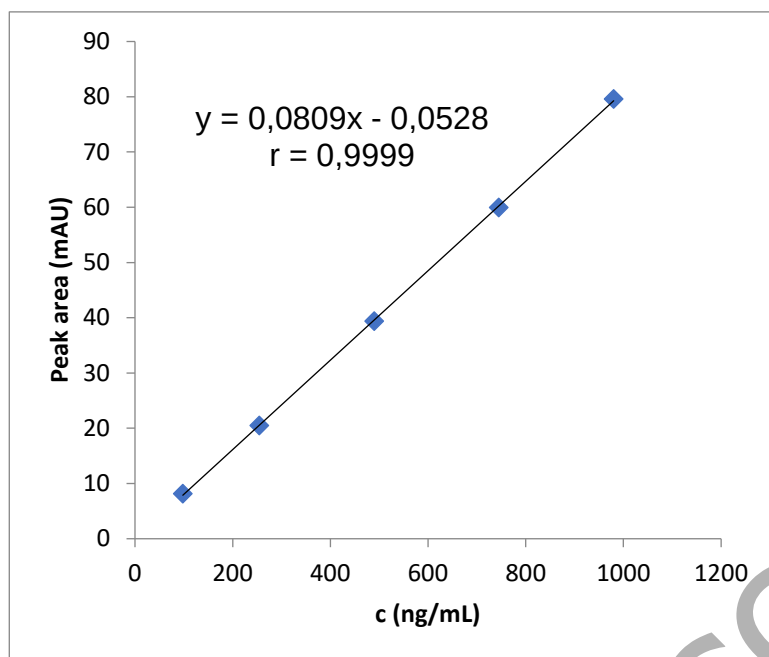


Figure 5. The calibration curve for impurity A

The obtained correlation coefficient value of $r=0.9999>0.9990$, along with a p-value of 0.86 ($p>0.05$), confirms the linearity of the method within the specified concentration range.

Method precision

The method precision was tested at three levels:

1. Injection repeatability
2. Repeatability of the method (intra-assay precision);
3. Intermediate precision.

Injection repeatability

To evaluate injection repeatability, a standard solution of impurity A at the working concentration was prepared and injected into a conditioned HPLC system. Based on the obtained peak area values of impurity A, the relative standard deviation (RSD) was calculated, which was then used to assess injection repeatability.

Based on the obtained RSD value ($0.65\% < 1\%$), it can be concluded that the method meets the requirements for injection repeatability.

Repeatability of the method (intra-assay precision)

To evaluate method repeatability, nine standard solutions were prepared—three solutions at each of three different concentrations—and injected into a conditioned HPLC system. Based on the obtained peak area values, the relative standard deviation (RSD) was calculated to assess the method's repeatability.

Based on the obtained RSD values (0.09% to 0.98% < 2%), it can be concluded that the method meets the requirements for repeatability.

Intermediate precision

Intermediate precision was evaluated by a second analyst on a different day, who independently prepared standard solutions of impurity A at three different concentrations, injected, and analyzed them under the specified chromatographic conditions.

The RSD values were below 3%, meeting the requirements for intermediate precision of the method.

Based on the obtained RSD values for injection repeatability (< 1%), repeatability ($\leq$ 2%), and intermediate precision ($\leq$ 3%), it can be concluded that the proposed analytical RP-HPLC method is precise.

Accuracy

To evaluate method accuracy, standard solutions of impurity A and laboratory mixture solutions containing the placebo and the standard substance were prepared at three concentrations: 80%, 100%, and 120% relative to the working concentration of 490 ng/mL. Each solution was injected three times into a conditioned chromatographic system. The results are expressed as the percent recovery of the known added amount of the analyte under investigation (Table 2).

Table 2. Data for evaluation of method accuracy

Solution label	Concentration C (ng / mL)	Peak area of impurity A from laboratory mixture	Standard peak area of impurity A	Measured concentration (ng / mL)	Recovery (%)
80 %		24.20	24.2	400	100.00
	400	24.50	24.8	395.16	98.78
		24.30	23.9	406.69	101.67
Average value		24.30	24.3	400.62	100.15
Sd					1.45
RSD					1.45
100 %		30.20	30.80	490.26	98.05
	500	30.40	31.00	490.32	98.06
		30.30	30.30	500.00	100.00
Average value		30.3	30.7	493.53	98.70
Sd					1.12
RSD					1.13
120 %		36.80	36.50	604.92	100.82
	600	36.20	36.60	593.46	98.91
		36.00	36.40	593.40	98.90
Average value		36.33	36.53	597.26	99.54
Sd					1.11
RSD					1.11

The obtained recovery values for all three tested concentration levels ranged from 98.70% to 100.14%, meeting the accuracy requirements for the assay method (98%–102%).

Limit of detection (LOD)

The LOD value was calculated from the standard deviation of the detector response, S_a , and the slope of the calibration curve using the following expression (1):

$$\text{Limit of detection (LOD)} = \frac{3,3S_a}{a} \quad (1)$$

The statistically determined limit of detection for impurity A was 18.52 ng/mL.

Limit of quantification (LOQ)

The LOQ value was calculated from the standard deviation of the detector response, S_a , and the slope of the calibration curve using the following expression (2):

$$\text{Limit of quantification (LOQ)} = \frac{10 \times S_a}{a} \quad (2)$$

The statistically determined limit of quantification for impurity A was 56,13 ng/mL.

Robustness

To evaluate the robustness of the method, deliberate minor changes in experimental conditions were applied:

1. pH value of the phosphate buffer.
2. the proportion of acetonitrile in the mobile phase;

The effect on the quantitative parameter, i.e., the peak area, was monitored. The results are presented in Tables 3 and 4.

Table 3. Data for evaluation of method robustness - Effect of mobile phase pH on the retention parameters of diltiazem and impurity A

Mobile phase: Buffer: acetonitrile 70 : 30 (V/V)			
flow rate: 1.2 mL/min, T25 °C			
pH	3.2	3.4	3.7
tr_{diltiazem} (min)	5.65	5.65	5.75
tr_{impurity A} (min)	4.55	4.55	4.55
t₀ (min)	1.55	1.55	1.55
k'_{diltiazem}	2.65	2.65	2.71
k'_{impurity A}	1.94	1.94	1.94
α	1.37	1.37	1.40

Table 4. Data for evaluation of method robustness - Effect of mobile phase composition on the retention parameters of diltiazem and impurity A

Mobile phase: Buffer: acetonitrile pH 3.4 flow rate: 1.2 mL/min, T25 °C			
% acetonitrile	28	30	32
tr_{diltiazem} (min)	7.93	5.65	4.21
tr_{impurity A} (min)	6.10	4.55	3.46
t₀ (min)	1.75	1.55	1.34
k'_{diltiazem}	3.53	2.65	2.14
k'_{impurity A}	2.49	1.94	1.58
α	1.42	1.37	1.35

The obtained results (Table 3) indicate that there are no significant changes in the retention parameters, including the retention factor (k') or the selectivity factor (α), allowing the conclusion that the method is robust to small variations in the buffer pH. Changes in mobile phase composition resulted in variations in the retention parameters, but not in the selectivity factor (Table 4), indicating that the method is also robust to minor changes in the mobile phase composition.

Determination of impurity A in diltiazem tablets

The validated chromatographic method was used to determine the content of the impurity A in diltiazem tablets. Standard and sample solutions were injected three times separately into a conditioned HPLC system.

The content of impurity A in diltiazem tablets was calculated using the following formula (3):

$$\% \text{ impurity A/tablet} = \frac{P_a \times C_x \times AM \times V}{P_{st} \times W_a \times DC} \quad (3)$$

Where:

- PaP – peak area of impurity A in the analysis
- Pst – peak area of the diltiazem standard
- Wa – measured mass of the sample, in grams
- AM – average tablet mass, in grams

- C – concentration of impurity A standard in the standard solution used for determination, in µg/mL
- DC – declared content of diltiazem in the tablets, in µg
- V – volume of the flask in which the sample was dissolved, in mL

The determination results are presented in Table 5 and Figure 6.

Table 5. Determination of impurity A

Declared content of diltiazem (90 mg / tablet)	Peak area	Measured concentration	
		(ng / tablet)	%
	11.61	160.59	0.0160
	11.57	160.04	0.0160
	11.56	159.90	0.0159
	11.52	159.35	0.0159
	11.81	163.36	0.0163
	11.80	163.22	0.0163
	11.62	160.73	0.0161
	11.58	160.18	0.0160
	11.61	160.59	0.0161
Average value	11.63	160.88	0.0161
SD			0.0002
RSD (%)		0.93	

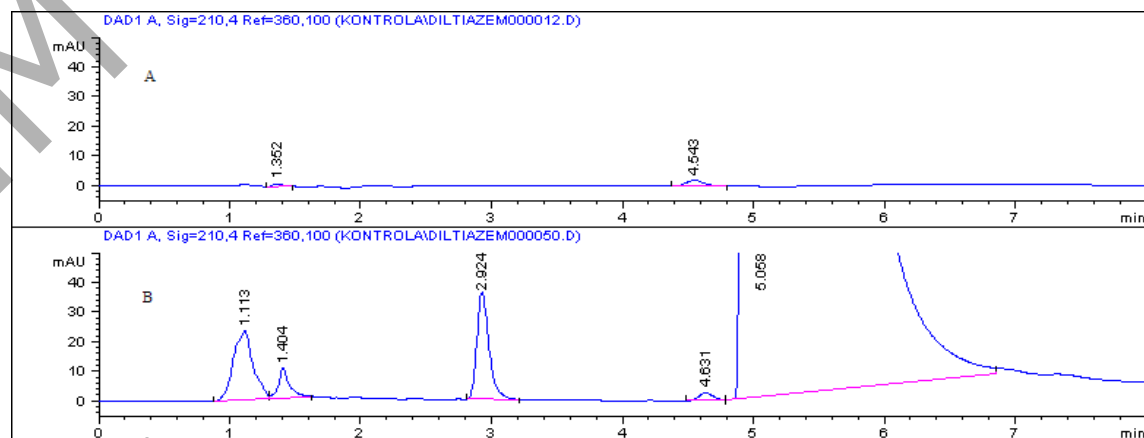


Figure 6. Chromatograms of impurity A standard (c = 490 ng/mL) and impurity A from diltiazem tablets

The developed method offers several advantages for routine pharmaceutical analysis. Unlike some previously reported approaches that were primarily focused on the determination of diltiazem and a broader range of related substances, the proposed method was specifically optimized and validated for the quantitative determination of impurity A in diltiazem tablets. The use of a conventional RP-HPLC system with an isocratic mobile phase consisting of phosphate buffer and acetonitrile enables a simple and practical analytical procedure. Furthermore, the method demonstrated satisfactory selectivity, precision, accuracy, sensitivity, and robustness, confirming its suitability for routine quality control applications.

The European Pharmacopoeia (6), in the monograph Diltiazem Hydrochloride, specifies a limit of 0.3% for impurity A, while USP 30 (7) specifies limits for Diltiazem Tablets of 0.5% for individual impurities and 1% for total impurities.

The determined content of impurity A was below the specified limits in both pharmacopoeias, amounting to 0.016%, indicating that the tested tablets comply with the monograph requirements regarding impurity A content.

Conclusion

A validated RP-HPLC method was developed for the determination of impurity A in diltiazem tablets. The method was validated in accordance with International council for harmonisation of technical requirements for pharmaceuticals for human use (ICH) guidelines (8). The method demonstrated linearity (98–980 ng/mL), satisfactory precision (RSD <1–3%), and accuracy (recovery 98.70–100.14%). LOD and LOQ were 18.52 ng/mL and 56.13 ng/mL, respectively. Robustness was confirmed, as minor variations in mobile phase composition and buffer pH did not affect retention or selectivity. The method reliably quantified impurity A in diltiazem tablets, meeting both USP monograph and manufacturer specification limits.

References

1. Institute for Medicines and Medical Devices. Summary of product characteristics. Aldizem, extended-release tablets, 90 mg, Alkaloid [Internet]. Montenegro: Institute for Medicines and Medical Devices; 2025 [cited 2026 Feb 25]. Available from: <https://cinmed.me/humani-lijekovi/humani-lijek/?id=2124>
2. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 39186, Diltiazem [cited 2026 Feb 27]. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Diltiazem>
3. Quaglia MG, Donati E, Fanali S, Bossù E, Montinaro A, Buiarelli F. Analysis of Diltiazem and its related substances by HPLC and HPLC/MS. *J Pharm Biomed Anal.* 2005;37:695–701. doi:10.1016/j.jpba.2004.11.050
4. Chatpalliwar VA, Porwal PK, Upmanyu N. Validated gradient stability indicating HPLC method for determining Diltiazem Hydrochloride and related substances in bulk drug and novel tablet formulation. *J Pharm Anal.* 2012;2(3):226–237. doi:10.1016/j.jpha.2012.01.003
5. Simultaneous enantioseparation of cis-diltiazem hydrochloride and its metabolite cis-desacetyldiltiazem using high performance liquid chromatography and capillary electrophoresis. *J Chromatogr A.* 2001;917(1–2):123–132.
6. European Pharmacopoeia Online. Monograph of Diltiazem hydrochloride. Implementation date 01/01/2017 (9.0). Text number 1004. Strasbourg: Council of Europe; 2026.
7. United States Pharmacopeia. Monograph of Diltiazem hydrochloride tablets. USP43–NF38. Rockville: United States Pharmacopeial Convention; 2020. p. 1406.
8. ICH Q2(R2) Guideline on validation of analytical procedures. EMA/CHMP/ICH/82072/2006. Committee for Medicinal Products for Human Use [Internet]. London: EMA; 2023 [cited 2026 Feb 13]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q2r2-guideline-validation-analytical-procedures-step-5-revision-1_en.pdf