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Quantification of Lorazepam using HPLC technique

Adnan Hamed Dhari[1, a)] Imad Tariq Hanoun[, b)]
[1] *University of Samarra, College of Education, Department of Chemistry, Samarra, Iraq.*
[a)] *Corresponding author*

Abstract :

LZM was successfully estimated using a high-performance liquid chromatography (HPLC) method, where optimal conditions were selected for the best results. The optimal conditions included using an L3-Si separation column (15 cm x 4.6 mm x 5 µm), a flow rate of 1.0 ml/s, a mobile phase pH of 3.0, a temperature of 30°C, and a detection wavelength of 228 nm. A calibration curve was constructed for the drug, showing linearity in the concentration range of 10-30 µg/ml. The coefficient of determination (R^2) reached 0.9991, with a limit of detection (LOD) of 0.1989 µg/ml and a limit of quantification (LOQ) of 0.6630 µg/ml. The percentage recovery values of the drug ranged from 97.479% to 100.881%, and the relative standard deviation did not exceed 0.9217%.

Keywords: Lorazepam, HPLC, Quantification.

التقدير الكمي لعقار اللورازيبام
بتقنية كروماتوغرافيا السائل عالي الأداء

عدنان حامد ضاري , عماد طارق حنون
جامعة سامراء / كلية التربية , قسم الكيمياء , سامراء- العراق

مستخلص:

تم تقدير LZM بنجاح بطريقة كروماتوغرافيا السائل عالي الأداء حيث تم اختيار الظروف التي اعطت افضل النتائج وبعد تثبيت الظروف الفضلى للطريقة المقترحة من عمود فصل من نوع L3 - Si (15 cm x 4.6 mm x 5µ) ومعدل جريان 1.0 مل/ ثانية وعند أس هيدروجيني للطور المتحرك pH=3.0 وبدرجة حرارة 30 C^o وعند طول موجي 228 نانومتر تم بناء منحني المعايرة للعقار، إذ تراوحت مدى خطية التراكيز لعقار الـ LZM بين (10-30) مايكروغرام/ مل.حيث بلغت قيمة معامل التقدير 0.9991 و كان حد الكشف (LOD) 0.1989 مكغم \ مل وحد التقدير (LOQ) 0.6630 مكغم \ مل وكانت قيم الاسترادادية المئوية للعقار ما بين (97.479٪-100.881٪) وقيم الانحراف القياسي النسبي لا تتجاوز 0.9217٪.

الكلمات المفتاحية: اللورازيبام ، كروماتوغرافيا السائل عالي الأداء ، التقدير الكمي .

Introduction

High-performance liquid chromatography (HPLC), also referred to as high-pressure liquid chromatography, is a widely used technique in biochemistry and analytical chemistry for the separation, identification, and quantification of active chemical compounds. This advanced method is designed to analyze complex mixtures by discerning and measuring each component [1,2].In HPLC, the solvent flows through the column under high pressure, reaching levels of up to 400 atm rather than relying solely on gravity. This elevated pressure enhances the separation of sample components based on their varying affinities [3].

Lorazepam is a benzodiazepine medication approved by the U.S. Food and Drug Administration (FDA) for alleviating symptoms of anxiety associated with anxiety disorders and anxiety-related insomnia. It is often preferred in hospital settings due to its rapid onset of action. Some of the common uses for lorazepam include the rapid sedation of agitated patients, treatment of delirium and alcohol withdrawal syndrome, management of insomnia and panic disorder, as well as alleviating nause [4].

The chemical name of the drug is 7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-1,4-benzodiazepin-2-one. Its molecular weight is 321.2 g/mol, and its molecular formula is $C_{15}H_{10}C_{l2}N_2O_2$. The drug appears as a white, nearly odorless powder that is insoluble in water and slightly soluble in both alcohol and chloroform. It has a melting point ranging from 166 to 168 degrees Celsius. The structural formula is illustrated in Figure 1[5].

Lorazepam binds to benzodiazepine receptors on neurons associated with gamma-aminobutyric acid (GABA-A) chloride channels at various sites within the central nervous system (CNS). This medication enhances the inhibitory effects of GABA by increasing the flow of chloride ions into the cells. The influx of chloride ions leads to hyperpolarization and stabilization of the cell membrane, while the increased inhibitory activity in the cerebral cortex is beneficial for managing seizure disorders[6].The drug was estimated by different methods, including spectroscopy [7], HPLC [8-9], and electrical methods [10-12].

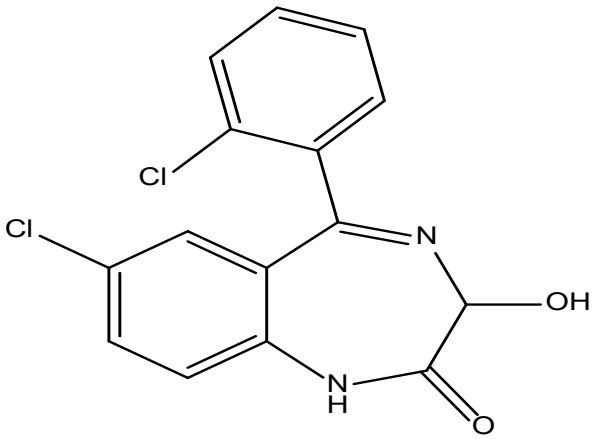

Figure 1 Structural formula of lorazepam

Experimental part

Instrumentals used

Table (1) shows the names of the devices used in the practical part of the thesis, their origin and manufacturer.

Table 1 Devices used

No.	Instrument	Model	Origin
1	HPLC	Shımadzu	Japan
2	Uv/Vis Spectrophotometer	Shımadzu	Japan
3	Sensitive Balance (four digits)	Sartorius Lab–BL	Germany
4	Ultrasonic	PTY. LTD. SYDNEY	Australıa
5	pH meter	JENWAY	United Kingdom

Chemical Materials Used

The solid and liquid chemicals listed in Table (2) were used in developing the proposed methods for the determination of LZM.

Table 2 Chemicals used

No.	Chemical material	Chemical formula	M.wt (g/mole)	Purity%	Company
1	Acetic acid	$C_2H_4O_2$	60.05	99.9%	Sigma -Aldrich
2	Acetonitrile	$C_2H_3N_1$	41.05	99.9%	BDH
4	Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	321.16	99.9%	SDİ
5	Sodium hydroxide	NaOH	40	97.5%	Sigma -Aldrich

Selective of Solvent

Multiple solvents were tested to dissolve the LZM drug. Ultimately, a solvent composed of methanol and water in a 75:25 (v/v) ratio was selected, as it achieved complete dissolution of the drug. This solvent was adopted for the proposed method.

Preparation of Solutions

A standard solution of lorazepam (LZM) with a concentration of 20 µg/ml

was prepared using the following procedure. First, 20 mg of lorazepam was accurately weighed and placed in a 100 ml volumetric flask. A small amount of a solvent mixture, consisting of methanol and water in a ratio of 75:25 (v/v), was added to dissolve the lorazepam. The volumetric flask was then subjected to ultrasonic treatment to eliminate any bubbles. The flask was filled to the 100 ml mark with the same solvent to produce a stock solution of lorazepam at a concentration of 200 µg/ml. Next, 1 ml of this stock solution was withdrawn and transferred to a 10 ml volumetric flask, which was then filled with the solvent to achieve a final standard solution concentration of 20 µg/ml.

Sodium hydroxide solution of approximate concentration (0.01 M)

The concentration was prepared from the base by weighing 0.4 g of sodium hydroxide and dissolving it in distilled water. The solution was then brought up to the mark with the same solvent in a 100 g volumetric flask to create a 0.1 molar sodium hydroxide solution. After that, 1 ml of this solution was withdrawn and diluted to a total volume of 10 ml with distilled water.

An acetic acid solution with an approximate concentration of 0.01 molar

was prepared as follows: First, 5.74 mL of acetic acid with a concentration of 17.4 molar was withdrawn and diluted to 100 mL with distilled water in a 100 mL volumetric flask to achieve a concentration of 1 molar. Then, 1 mL of this 1 molar solution was further diluted to 100 mL with distilled water.

Preliminary tests

LZM was estimated using the HPLC technique after testing several solvents to determine the most suitable one for this drug. The wavelength was fixed

by performing a spectral scan of the drug solution. Following this, the drug was injected with a volume of 20 microliters at a concentration of 20 micrograms per milliliter. Various mobile and stationary phases were evaluated to establish the optimal method for separating and selectively identifying the drug. The requirements for separation efficiency in high-performance liquid chromatography were also taken into account.

Preparation of mobile phase

Mixtures with different proportions of mobile phase components were prepared in order to select the best of them in terms of chromatographic separation and peak areas of the drug. Several mobile phases were prepared and the best of them in terms of chromatographic separation and peak areas were selected. The selected mobile phase consisted of water, acetonitrile and glacial acetic acid in proportions of (50:50:0.4) v/v/v, respectively. This mobile phase gave good separation and sharp and narrow peaks of the drug compared to other mobile phases, so it was adopted as a mobile phase for the proposed method. The mobile phase was placed in an ultrasonic device to get rid of bubbles and filtered with filter paper.

Results and discussion

Establishing the best conditions

Choose the Wavelength

The spectral scan of the drug solution revealed that the maximum wavelength for the highest absorption of the drug occurred at 228 nm, as illustrated in Figure 2.

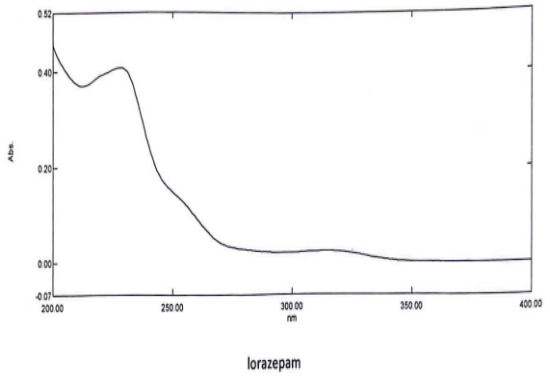

Figure 2 Lorazepam spectrum

Selection of Mobile Phase

Mixtures with various proportions of the mobile phase components were prepared to determine the optimal conditions for chromatographic separation and the peak areas of the drug. The drug was injected at a concentration of 20 µg/ml, with a flow rate of 1 ml/min,

at laboratory temperature, using an L3-Si separation column (15 cm x 4.6 mm x 5 µm) at a wavelength of 228 nm. A syringe with a volume of 20 µl was utilized for the injections.The mobile phase, consisting of water, acetonitrile, and glacial acetic acid, was selected in the proportions of 50:50:0.4 (v/v/v) because it provided the best chromatographic separation, tailing factor, theoretical plate number, theoretical plate equivalent height, and yielded the highest peak areas of the drug compared to the other tested proportions. The mobile phase was placed in an ultrasonic device to remove bubbles and then filtered using filter paper.

Selection a separation column

.The selection of the separation column is based on the type of material being separated. When choosing the appropriate column, it is essential to consider its efficiency, which is related to the number of theoretical plates (N). A higher N value indicates narrower peaks and closer adherence to ideal behavior. The number of theoretical plates is influenced by how the column is prepared and the nature of the material being separated. Therefore, the optimal separation column has a high N value, a large peak area, a small height equivalent to a theoretical plate (H), and a tailing factor (Tf) of less than 1.5. To identify the most suitable separation column, the LZM drug was injected at a concentration of 20 µg/ml. The mobile phase consisted of water, acetonitrile, and glacial acetic acid in a ratio of 50:50:0.4 (v/v/v). The analysis was conducted using a wavelength of 228 nm, a flow rate of 1 ml/min, and a syringe volume of 20 µl. Four separae tion columns were evaluated, as shown in Table 3. The L3-Si column (15 cm x 4.6 mm x 5 µ) was selected because it provided an acceptable number of theoretical plates, a suitable height equivalent to a theoretical plate (H), and an appropriate peak area for the LZM drug. The other columns were not chosen due to their unsatisfactory chromatographic separation, even though they exhibited higher peak areas and a greater number of theoretical plates than the selected column.

Table 3 Chapter Columns

Column	Materials	Peak Area (mv)	tR (min)	N	H
L1 C18 (15 cm x 4.6 mm x 5µ)	LZM	3636813	3.176	4806	31.21
L3 Si (15 cm x 4.6 mm x 5µ)	LZM	3114102	3.141	6685	22.438
L7 C8 (15 cm x 4.6 mm x 5µ)	LZM	3734312	6.219	6791	22.088
L11 Phenyl (15 cm x 4.6 mm x 5µ)	LZM	3395702	3.420	7587	19.770

As shown in the following figures (3)–(6):

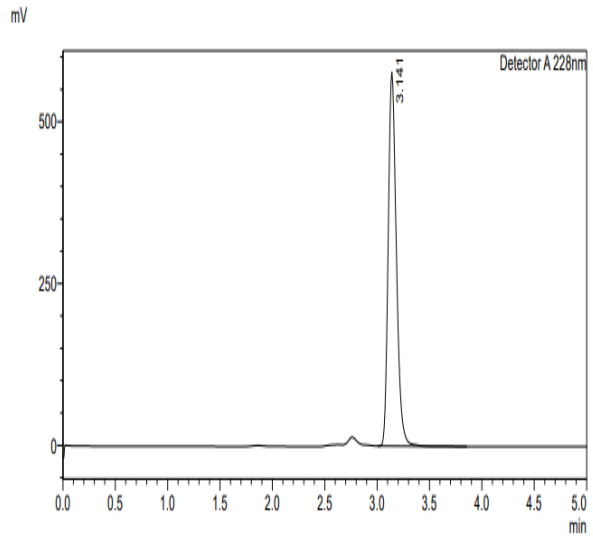

Figure 3 Chromatogram of the L3 columnpH Effect

The study investigated the impact of varying the pH of the mobile phase on the retention time and peak area values for a range of pH levels (3.0, 3.5, 4.0). Sodium hydroxide (0.01 M) and acetic acid (0.01 M) were used in the mobile phase. The drug was injected at a concentration of 20 µg/ml with a flow rate of 1 ml/s at laboratory temperature, utilizing an L3-Si separation column (15 cm x 4.6 mm x 5 µm). The detection was performed at a wavelength of 228 nm with a syringe volume of 20 µl. The optimal results were observed at a pH of 3.0, which was selected for the mobile phase due to its favorable values for the number of theoretical plates, equivalent height of theoretical plates, and an acceptable peak area for the drug compared to other pH levels. This data is summarized in Table 4 and illustrated in Figures 4.

Table 4 Effect of pH

pH	material	Peak Area (mv)	tR (min)	N	H
3.0	LZM	3075888	3.214	5555	27
3.5	LZM	3153909	3.208	5726	26.19
4.0	LZM	3048073	3.207	5768	26

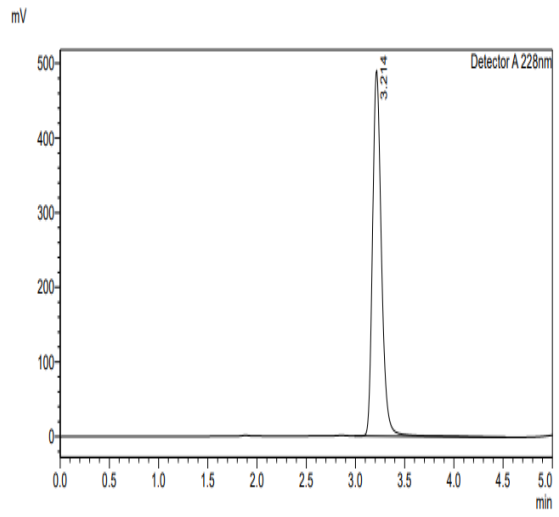

Figure 4 Chromatogram of LZM at pH 3.0

Selection the Temperature

The drug was injected at a concentration of 20 µg/ml with a flow rate of 1 ml/s, using an L3-Si separation column (15 cm x 4.6 mm x 5 µ). The detection wavelength was set at 228 nm, and the syringe volume used was 20 µl. The mobile phase had a pH of 3.0, and various temperatures were tested, as detailed in Table 5 and Figures 5. The results indicated that the highest peak area of the drug occurred at a temperature of 30°C, which also corresponded to the highest number of theoretical plates and the lowest equivalent height of the theoretical plate. Therefore, this temperature was selected for further analysis.

Table 5 Selection of column temperature

Temp.	Material	Peak Area (mv)	tR (min)	N	H
25 C°	LZM	3075888	3.214	5555	27
30 C°	LZM	3007884	3.210	5169	29.019
40 C°	LZM	2999285	3.160	---	---
50 C°	LZM	7075135	4.296	---	---

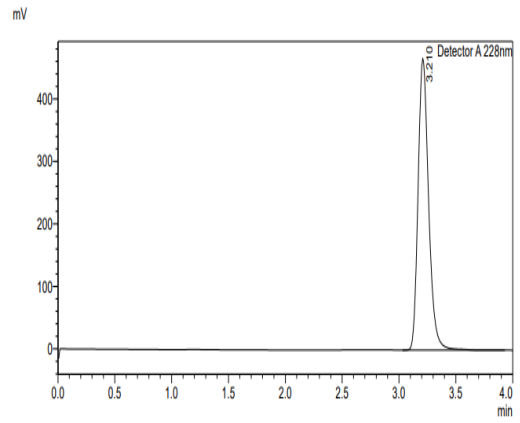

Figure 5 Chromatogram of LZM at 30°C

Selection the flowrate

The drug solution was injected at a concentration of 20 µg/ml using a syө ringe volume of 20 µl. The experiment was conducted under fixed conditions, including the column type, temperature, mobile phase, wavelength, and acidity of the mobile phase while varying the flow rates between 1.5, 1.0, 0.7, and 0.5 ml/s. It was observed that a flow rate of 1.0 ml/s produced an acceptable peak area for the drug, along with suitable values for the number of theoretical plates and the equivalent height of the theoretical plate. Therefore, this flow rate was adopted for further analysis, as detailed in Table 6 and chromatograms 14 to 17 for each flow rate.

Table 6 Flow rates

Flow rate	Material	Peak Area (mv)	tR (min)	N	H
0.5 mL/min	LZM	6107659	6.419	7428	20.19386
mL/min 0.7	LZM	4384497	4.588	6667	22.49888
mL/min 1.0	LZM	2938970	3.212	5151	29.12056
mL/min 1.5	LZM	2024945	2.159	3279	45.74565

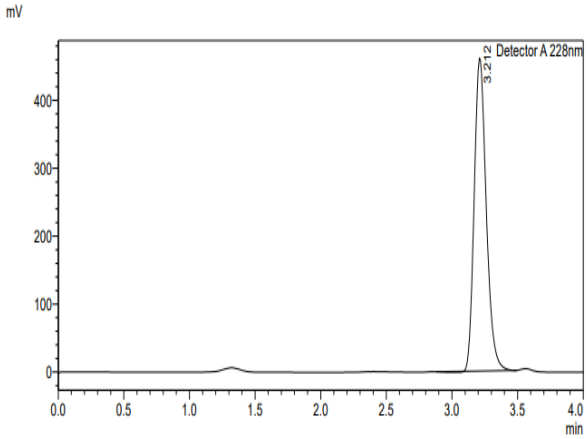

Figure 6 Chromatogram of LZM drug at a flow rate of 1.0 mL/s

Establishing a calibration curve

A series of LZM drug concentrations were prepared after establishing the optimal conditions for the proposed method. The separation was conducted using an L3 - Si column (15 cm x 4.6 mm x 5 µm) with a flow rate of 1.0 ml/s, under a mobile phase pH of 3.0, at a temperature of 30°C and a detection wavelength of 228 nm. A

calibration curve for the drug was created, with the linearity range for LZM concentrations spanning from 10 to 30 micrograms/ml. These concentrations were prepared in 10 ml volumetric flasks. The calibration curve was plotted by placing the areas of the peaks on the Y-axis and the corresponding concentrations on the X-axis, as shown in Figure 18. The correlation coefficient (R^2) for the calibration curve was determined to be 0.9991.Table 7 presents the peak area values for the LZM concentrations, while Figures 19, 20, 21, and 22 illustrate the chromatograms corresponding to the studied concentrations of LZM.

Table 7 Peak areas Concentration range of calibration curve

Conc. (µg/mL)	Peak Area (mv)	tR (min)
10	1519016	3.199
15	2322078	3.197
20	3053916	3.193
25	3793495	3.181
30	4467917	3.179

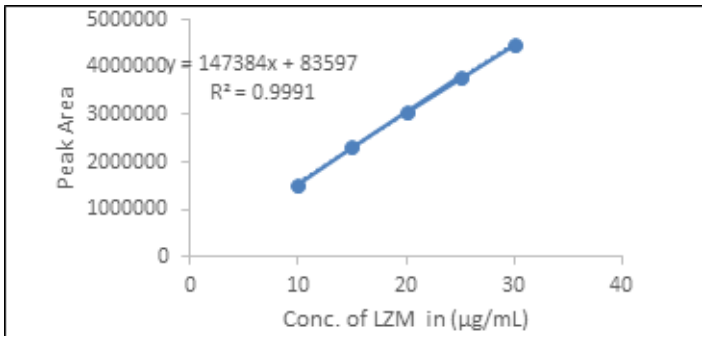

Figure 7 Calibration curve for LZM

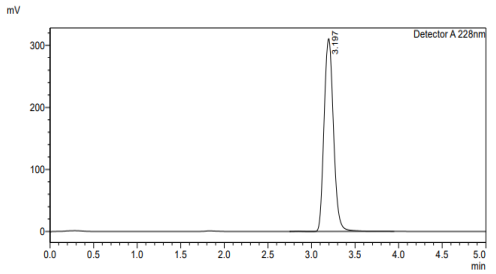

Figure 9 Chromatogram of LZM drug at a concentration of 15 µg/ml

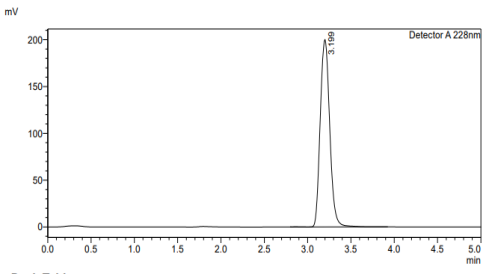

Figure 8 Chromatogram of LZM at a concentration of 10 µg/ml

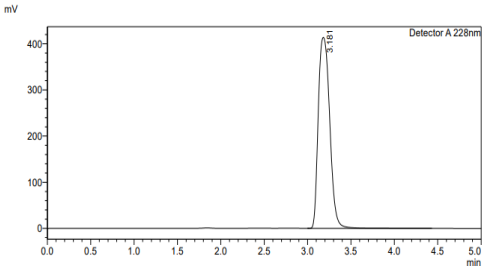

Figure 11 Chromatogram of LZM at a concentration of 25 µg/ml

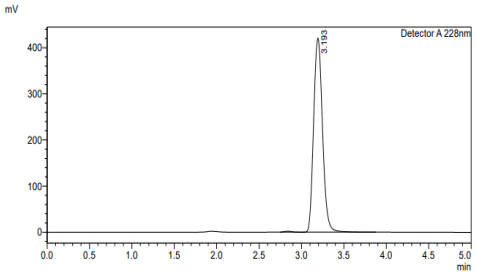

Figure 10 Chromatogram of LZM at a concentration of 20 µg/ml

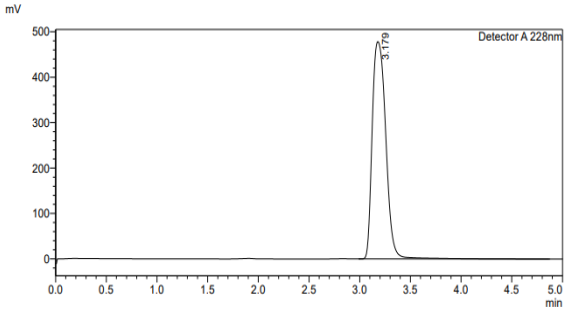

Figure 12 Chromatogram of LZM at a concentration of 30 µg/ml

Accuracy and Precision

A study was conducted to evaluate the accuracy and consistency of the proposed method. This assessment was performed by calculating the percentage recovery value (Rec%) to express the accuracy of the results and the relative standard deviation (RSD%) to indicate the consistency of the results for the drug at three different concentrations along the calibration curve. Each concentration was measured three times. The percentage recovery values for the drug ranged from 97.479% to 100.881%, and the relative standard deviation values did not exceed 0.9217%. These results indicate that the method demonstrates high accuracy and consistency, as shown in Table 8.

Table 8 Relative standard deviation and percent recovery values

Drug	Conc.taken (µg/mL)	Peak Area (mv)	Conc.found (µg/mL)	Rec.%	RSD%*
LZM	10	1520293	9.747	97.4797	0.6428
LZM	20	3057269	20.176	100.8818	0.253
LZM	30	4432533.5	29.507	98.3584	0.9217

* Average of three readings

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined using the areas of the peaks corresponding to the lowest concentrations from the drug's calibration curve, as shown in Table 9.

Table 9 Detection limit and quantitation limit

Drug	Conc.taken (µg/mL)	SD*	Slope	LOD	LOQ
LZM	10	9772.883	147384	0.1989	0.6630

*Average of three readings

Application of method

After determining the optimal conditions for the proposed method, a single concentration of the drug LZM was prepared and injected from the pharmaceutical formulation Lorazepam using the concentrations studied in this method. The application of the straight-line equation for the drug indicated that the proposed method demonstrates high accuracy and consistency, as shown by the results in Table 10.

Table 10 Application of the proposed method for LZM in the pharmaceutical preparation Lorazepam

Drug	Conc. taken (µg/mL)	Peak Area (mv)	tR (min)	Conc. Founded (µg/mL)	Rec%	RSD%*
LZM	20	3004150	3.212	19.81	99.05%	0.514

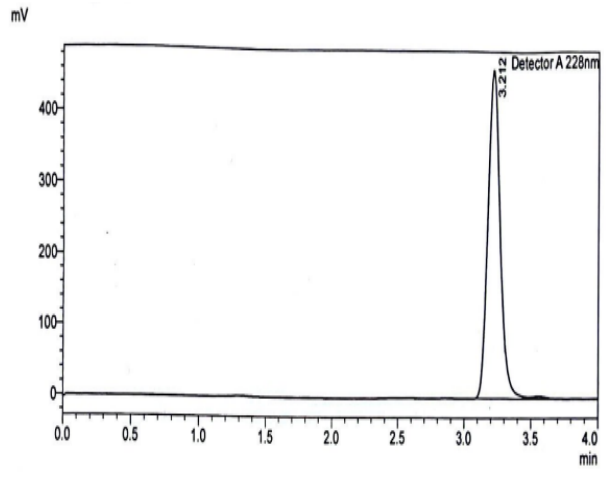

Figure 13 LZM drug at a concentration of 20 µg/ml

Compare method

Table 11 compares the results of the proposed method with those of another method to demonstrate its efficiency. The findings indicate that the proposed method outperforms the other method in several areas, including linearity, estimation coefficient, detection limit, quantitative limit, and retention time.

Table 11 Comparison of the results of the proposed method with other analytical methods

Parameters	Drug	Present method	Other method[13]
Linearity Range (µg/mL)	LZM	(10-30)	(40-500)
Correlation coefficient (R^2)	LZM	0.9991	0.999
LOD(µg/mL)	LZM	0.1989	0.927
LOQ(µg/mL)	LZM	0.6630	3.097
Rec% (µg/mL)	LZM	(97.4797-100.8818)	---
RSD%	LZM	(0.253-0.9217)	1.1%
tR (min.)	LZM	3.181	230nm

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