

Development and validation of a sensitive RP-HPLC method for quantifying oxaliplatin in ZIF-8 as a drug delivery system

Toan Quyen Pham^{1,2,3}, Xuan Hiep Nguyen^{1,2}, Nhat Hoang Pham^{1,2}, Linh Ho Thuy Nguyen^{1,2}, Minh-Tri Le^{1,2,3}, Tan Le Hoang Doan^{1,4,*}



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ABSTRACT

Introduction: Oxaliplatin is widely utilized in cancer therapy but is often limited by significant side effects. Zeolitic Imidazolate Framework-8 (ZIF-8), a type of metal-organic framework nanoparticle, has been investigated as a delivery system for oxaliplatin, offering advantages such as targeted cancer cell delivery and controlled drug release. This study aimed to develop and validate a sensitive, straightforward, and cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) method for quantifying oxaliplatin within ZIF-8. **Methods:** Various chromatographic conditions were developed to accurately quantify oxaliplatin-loaded ZIF-8. The final HPLC method was validated in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. **Results:** Chromatographic separation was achieved on an RP-C18 column (250 x 4.6 mm, 5 μ m) using isocratic elution with a methanol-water mobile phase (1:1 ratio) at a flow rate of 0.8 mL/min. Detection was performed with a PDA detector set at 230 nm. The method demonstrated suitability through validation of specificity, linearity, accuracy, precision, range, and robustness. Linearity was confirmed within a concentration range of 0.1-1.5 mg/mL, with a correlation coefficient (R^2) of 0.9994. Accuracy ranged from 98.79% to 99.71%, with a mean accuracy of 99.16%, and the relative standard deviation was 0.48%. **Conclusion:** The validated RP-HPLC method offers a reliable and robust means for assessing the loading capacity and release performance of oxaliplatin in ZIF-8, serving as an essential analytical tool for evaluating this drug delivery system. This approach supports precise formulation assessment, aiding in the accelerated development of future applications in drug delivery research.

Key words: Oxaliplatin, HPLC, quantitative evaluation, ZIF8 material, drug delivery systems

¹Vietnam National University Ho Chi Minh City, Ho Chi Minh city, Viet Nam

²University of Health Sciences, Vietnam National University Ho Chi Minh City, Ho Chi Minh City, Viet Nam

³Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam

⁴Center for Innovative Materials and Architectures, Ho Chi Minh City, Viet Nam

Correspondence

Tan Le Hoang Doan, Vietnam National University Ho Chi Minh City, Ho Chi Minh city, Viet Nam

Center for Innovative Materials and Architectures, Ho Chi Minh City, Viet Nam

Email: dlhtan@inomar.edu.vn

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INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, imposing a considerable economic burden on many nations¹. Chemotherapy, a primary approach to cancer treatment, relies on drugs to eliminate cancer cells, inhibit tumor growth, and prevent metastasis². Among these therapeutic agents, platinum-based compounds - particularly oxaliplatin (OXP) - are widely employed. However, oxaliplatin often necessitates high dosages and exhibits limited selectivity, which can compromise treatment efficacy and amplify adverse effects³. To overcome these limitations, metal-organic frameworks (MOFs) such as Zeolitic Imidazolate Framework-8 (ZIF-8) have been investigated as potential drug delivery systems. ZIF-8 shows promise for oxaliplatin encapsulation, offering benefits such as enhanced drug stability, improved bioavailability, and controlled release profiles⁴⁻⁷.

Previous studies have primarily utilized Inductively Coupled Plasma (ICP) analysis to measure drug loading onto nanomaterials, though this technique lacks

the specificity and sensitivity of High-Performance Liquid Chromatography (HPLC). Additionally, existing HPLC methods for quantifying free oxaliplatin or its injectable formulations are not directly applicable to oxaliplatin-loaded ZIF-8 (OXP@ZIF-8) due to the distinctive properties of this nanoparticle-based system. This highlights the need for a quantitative method that is straightforward, accurate, and reliable for determining the active pharmaceutical ingredient (API) in OXP@ZIF-8 and evaluating its release profile⁸. The aim of this study is to develop and validate an HPLC method specifically for quantifying oxaliplatin in ZIF-8 formulations, facilitating the assessment of drug loading and release efficiency - an approach not yet documented in the literature.

MATERIALS - METHODS

Materials

Chemical reagents and solutions: Oxaliplatin samples were generously provided by the Institute of Drug Quality Control, Ho Chi Minh City (Vietnam) (Lot

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No. CA-22060) as a gift, while the oxaliplatin European Pharmacopoeia standard was procured from SigmaAldrich (Germany) (Lot No. R152G0). ZIF-8 was synthesized following the protocol published by INOMAR (Vietnam). Methanol and water, both HPLC-grade, were obtained from Fisher Scientific (USA).

Instruments: A Shimadzu HPLC system LC-2030 3D equipped with a Photo Diode Array (PDA) detector (Japan) was utilized for the analysis. Data acquisition and processing were carried out using Lab Solution software (Japan). A Phenomenex Reverse-Phase C18 (RP-C18) column (250 x 4.6 mm, 5 μ m) (USA) was used, along with other precision analytical glassware for the study.

Preparation of solutions

Stock solution: An accurately measured 10.0 mg of oxaliplatin sample was dissolved in water in a 10 mL volumetric flask and diluted to the calibration mark.

OXP@ZIF-8 sample solutions: Precisely 5.0 mg of each oxaliplatin-loaded ZIF-8 complex was weighed into an amber-colored flask, followed by the addition of 5 mL of the stock solution. The mixture was stirred continuously at room temperature for 24 hours to ensure complete drug release. After centrifugation at 12,000 rpm for 10 minutes at 4°C, the supernatant was collected and filtered through a 0.45 μ m nylon filter before injection into the HPLC system.

Standard solution: An exact 10.0 mg of oxaliplatin standard was dissolved in water in a 10 mL volumetric flask and diluted to the mark. The solution was filtered through a 0.45 μ m nylon filter into a 1.5 mL vial.

The stock solution was prepared at a high concentration to maintain stability and facilitate serial dilutions for creating standard solutions with varying concentrations for the calibration curve. ZIF-8 samples (without oxaliplatin) were prepared under identical conditions to assess the specificity of the method.

HPLC parameters development

Determination of detection wavelength: The UV-Vis spectrum in HPLC chromatography was utilized to identify the wavelength at which oxaliplatin exhibited maximum absorbance.

Method development: Various mobile phase ratios were tested using an RP-HPLC column paired with a PDA detector. The optimal chromatographic conditions were determined by evaluating factors such as retention time, resolution, tailing factor, and peak purity.

Validation of analytical method

The developed HPLC with suitable criteria was validated according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R1) guidelines for analytical criteria⁹ such as system suitability, specificity, linearity, accuracy, precision, range, robustness. The limit of detection (LOD) and limit of quantitation (LOQ) have been calculated based on a signal-to-noise ratio ($S/N = 3$ for LOD and $S/N = 10$ for LOQ) as per ICH guidelines.

RESULTS

HPLC parameters development

Various chromatographic conditions, including different mobile phase ratios, flow rates, column temperatures, and detection wavelengths, were evaluated. The optimal conditions were determined to be a methanol/water ratio of 1:1, a flow rate of 0.8 mL/min, room temperature, and a detection wavelength of 230 nm. These conditions provided sharp, well-resolved peaks and reproducible retention times. The chromatograms showed a retention time (t_R) of 3.7 minutes for oxaliplatin, with well-defined peak characteristics (Figure 1).

Validation of analytical method

System suitability was evaluated by injecting a standard solution six times and recording the results. As shown in Table 1, the % Relative Standard Deviation (RSD) of the measured parameters met the acceptance criterion of $\leq 2.0\%$. Consequently, the method was deemed suitable for system performance.

Specificity

The analytical method's specificity - its ability to accurately measure the target compound in the presence of other components - was evaluated. Specificity testing was performed separately for oxaliplatin, ZIF-8, and their combination under standard conditions to ensure accurate identification. Additionally, peak purity was assessed to confirm the method's selectivity. Figures 2, 3, 4, 5 and 6 shows the chromatogram from the specificity study, demonstrating that oxaliplatin was completely separated from other components in the mixture. In the standard solution, a single oxaliplatin peak appeared at 3.7 minutes, while the blank chromatogram using the diluent showed no peaks. Chromatograms of ZIF-8 samples displayed additional peaks, but their retention times differed from that of oxaliplatin. In spiked standard samples, the oxaliplatin peak area and height increased

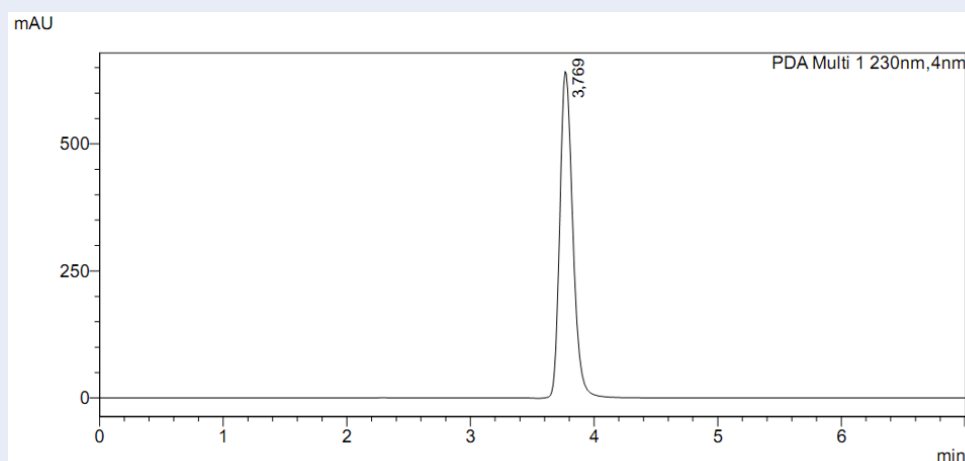


Figure 1: Chromatography of oxaliplatin standard; (Source: Authors' own work)

Table 1: System suitability results (n=6)

S.No	t_R (min)	Area (mAU*s)	Tailing factor	Resolution	Theoretical plate count
1	3.761	4692636	1.236	2.638	6535
2	3.764	4694008	1.243	2.641	6614
3	3.764	4690122	1.237	2.645	6470
4	3.764	4692585	1.234	2.648	6468
5	3.764	4691154	1.236	2.651	6532
6	3.764	4690837	1.242	2.649	6585
Mean	3.764	4691890	1.238	2.6445	6534
Acceptance criteria	-	-	0.8 – 1.5	≥ 1.5	> 2000
Standard deviation	0.001	1435.395	0.004	0.394	59.090
%RSD	0.033	0.030	0.294	0.19	0.904

[Data source: Experimental data generated by the authors using HPLC system LC-2030 3D; statistical parameters calculated using Microsoft Excel]

compared to the base sample, while other peaks remained consistent. The peak purity exceeded the required standard of 99.99%, confirming the analytical method's specificity.

Linearity

To evaluate the linearity of oxaliplatin in this method, a series of standard solutions at concentrations ranging from 0.1 to 1.5 mg/mL were prepared (Table 2). Each concentration was injected in triplicate under identical conditions, and the mean peak areas were used to construct the calibration curve. Linearity was assessed using the least squares linear regression method.

The linear equation for oxaliplatin was determined to be $\hat{y} = 5E+06x + 106071$, with a high goodness-of-

fit (R^2) value of 0.9994 (Figure 7), indicating a strong linear relationship between oxaliplatin concentration and peak area. This result confirms that the HPLC method meets the linearity requirement within the tested concentration range of 0.1 to 1.5 mg/mL for oxaliplatin.

Precision

Precision was evaluated through both repeatability and intermediate precision, using multiple measurements of the standard solution. Repeatability was assessed with six standard solutions at 100% concentration on the same day, while intermediate precision was determined with twelve standard solutions at 100% concentration, prepared by two different individuals on separate days. The Relative Standard De-

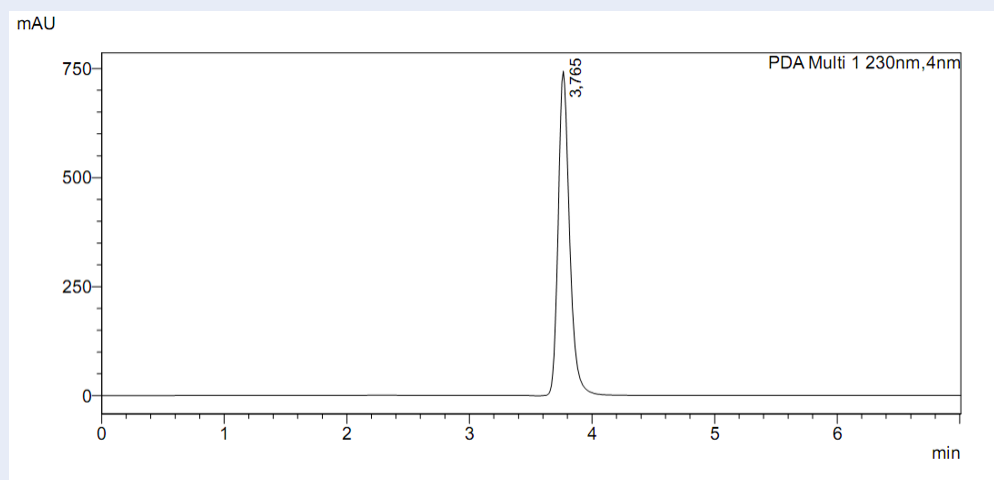


Figure 2: Chromatograms of the specificity resulting from standard solution; (Source: Authors' own work)

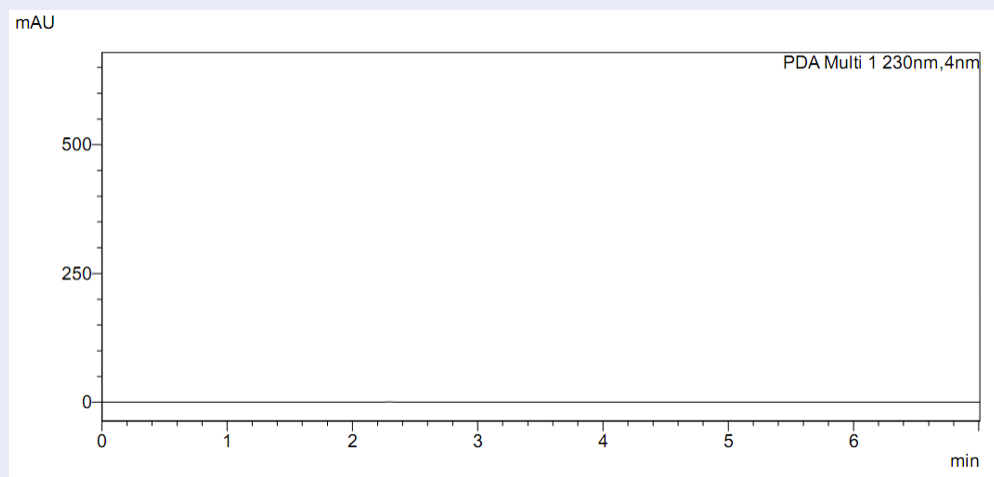


Figure 3: Chromatograms of the specificity resulting from blank solution; (Source: Authors' own work)

Table 2: Linearity results

Concentration (mg/mL)	Peak area (mAu*s)			Mean (mAu*s)	%RSD
0.1	487230	487230	486890	487116.7	0.040
0.2	1006939	1006217	1004721	1005950.0	0.112
0.4	2005273	2006859	2005937	2006016.0	0.039
0.5	2389135	2383821	2387734	2386897.0	0.115
0.8	3826695	3824610	3823250	3824852	0.045
1.0	4706463	4710812	4707608	4708294	0.047
1.2	5627255	5629375	5625684	5627438	0.032
1.5	6896038	6894973	6895497	6895503	0.007

[Data source: Experimental data generated by the authors using HPLC system LC-2030 3D; statistical parameters calculated using Microsoft Excel]

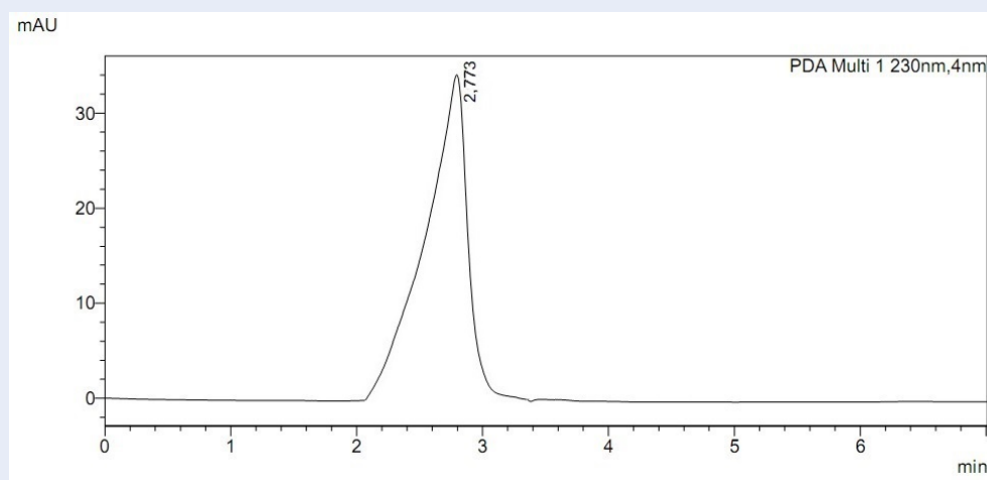


Figure 4: Chromatograms of the specificity resulting from ZIF-8 material; (Source: Authors' own work)

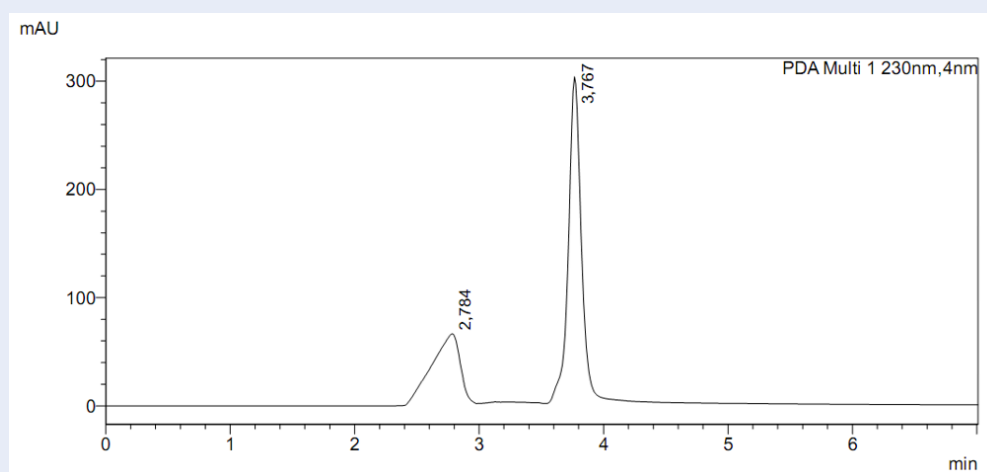


Figure 5: Chromatogram of oxaliplatin-loaded ZIF-8 standard (OXP@ZIF-8 standard); (Source: Authors' own work)

viation (RSD) of oxaliplatin content was calculated from the peak area results.

As shown in Table 3 and Table 4, the method met the precision criteria, with RSD values of 1.603% and 1.536%, both well below the 2% threshold. These results align with ICH Q2(R1) criteria for analytical precision. Analysis using the F-test and T-test revealed significant variance differences between the two individuals, but no significant difference in the mean values, with $F_{tn} < F_{0.01}$ and $T_{tn} < T_{0.01}$. Therefore, the HPLC method satisfied the precision requirements.

Accuracy

For accuracy evaluation, three standard solutions (80%, 100%, and 120%) were spiked into OXP@ZIF-8 sample solutions - by three different individuals at

separate times - to calculate recovery rates, ensuring the method's reliability for determining oxaliplatin content in the matrix. Each concentration was injected in triplicate. The percentage recovery of the added oxaliplatin and the Relative Standard Deviation (RSD) of the recovery rates were calculated from these results (Table 5).

The accuracy results showed percentage recovery values ranging from 98.35% to 99.97%, with a mean recovery of 99.26% and an RSD of 0.539% across all three concentration levels, confirming the method's reliability in accordance with ICH Q2(R1) guidelines. These findings indicate that the method can be reliably applied to determine oxaliplatin in a mixture with ZIF-8.

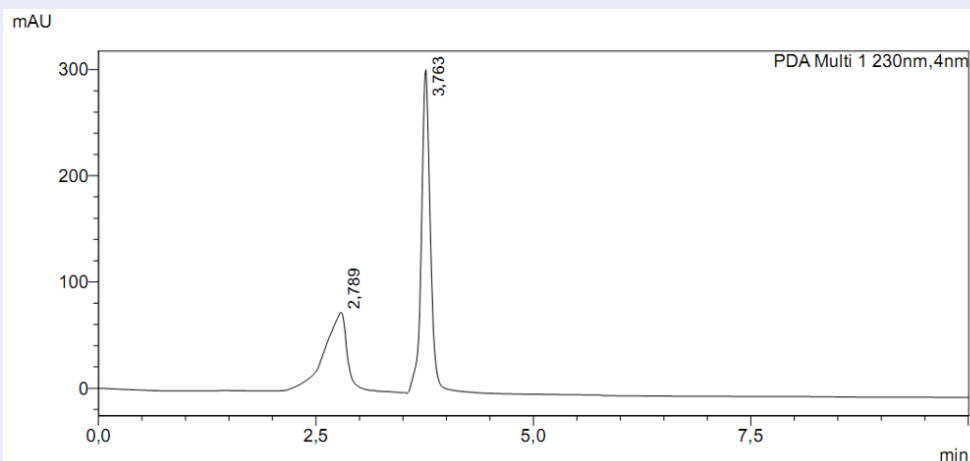


Figure 6: Chromatogram of oxaliplatin-loaded ZIF-8 sample (OXF@ZIF-8 sample); (Source: Authors' own work)

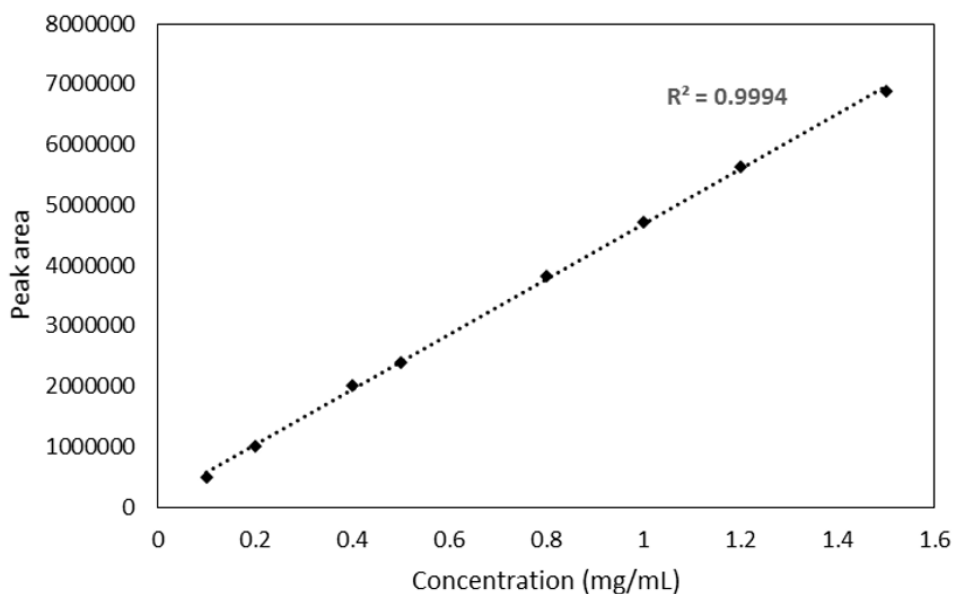


Figure 7: Correlation curve analysis for oxaliplatin regression; (Source: Authors' own work)

Range: The method's range was established at 0.1 to 1.5 mg/mL based on linearity, precision, and accuracy criteria. This range ensures reliable quantification, with a correlation coefficient (R^2) of 0.9994, accuracy between 98.35% and 99.97%, and precision with RSD below 2%, fully complying with ICH Q2(R1) guidelines.

Robustness: Several minor variations in experimental conditions were assessed by calculating the Relative Standard Deviation (RSD) for key parameters, including flow rate adjustments of ± 0.1 mL/min, column

temperature variations of $\pm 2^\circ\text{C}$, and different vial positions in the tray. The chromatographic results confirmed that the method satisfied the requirements for %RSD, tailing factor, and resolution.

The limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ were determined to be $0.0354 \mu\text{g/mL}$ and $0.118 \mu\text{g/mL}$, respectively, based on a signal-to-noise ratio (S/N).

Table 3: Repeatability results

Sample No.	Weight (mg)	Peak area (mAu*s)	Content (%)
1	10.0	4640436	98.559
2	10.0	4704070	99.910
3	10.1	4800651	101.961
4	10.0	4719347	100.234
5	10.0	4680966	99.420
6	10.0	4641964	98.591
	Mean (%)		99.779
	%RSD (n =6)		1.603

[Data source: Experimental data generated by the authors using HPLC system LC-2030 3D; statistical parameters calculated using Microsoft Excel]

Table 4: Intermediate precision results

Sample No.	Member 1			Member 2			
	Weight (mg)	Peak area (mAu*s)	Content (%)	Weight (mg)	Peak area (mAu*s)	Content (%)	
1	10.1	4780564	101.535	10.1	4789229	101.719	
2	10.1	4774908	101.415	10.1	4873749	103.514	
3	10.1	4785861	101.647	10.1	4819844	102.369	
4	10.0	4647844	98.716	10.1	4893462	103.932	
5	10.0	4648704	98.734	10.0	4672375	99.237	
6	10.1	4782363	101.573	10.1	4807782	102.113	
	Mean (%)		100.603	Mean (%)		102.148	
	%RSD (n =6)		1.448	%RSD (n =6)		1.624	
Average content (%) of two members (n = 12): 101.376 %RSD (n=12): 1.536							
$F_{in} = 1.297 < F_{0.01} = 10.967$							
$T_{in} = 1.714 < T_{0.01} = 3.196$							

[Data source: Experimental data generated by the authors using HPLC system LC-2030 3D; statistical parameters calculated using Microsoft Excel]

DISCUSSION

This analytical method employs reverse-phase chromatography with a PDA detector and an ODS-C18 column, developed based on the polarization characteristics of oxaliplatin. The carefully selected mobile phase, a 1:1 mixture of methanol and water, enhances the stability of the target compound while facilitating its complete isolation from other substances at a detection wavelength of 230 nm.

The method's effectiveness is demonstrated by its ability to selectively isolate oxaliplatin from other components, which is critical for accurately quantifying drug loading and monitoring release kinetics within the ZIF-8 drug delivery system. Its high sensitivity

and specificity provide precise measurements of oxaliplatin concentrations, minimizing potential interference from other system components. This method has been successfully applied in ZIF-8 analyses, underscoring its capability to enhance and refine drug delivery processes.

While HPLC remains the gold standard for quantifying oxaliplatin in commercial formulations, these methods are tailored for free oxaliplatin or injectable forms. When applied to nano-materials like ZIF-8, matrix interference and specific interactions between the drug and carrier compromise their accuracy. ICP-MS provides precise measurements of platinum content but lacks specificity for evaluating drug release

Table 5: Accuracy results

Level (%)	Weight added (mg)	Concentration added (mg/mL)	Mean of peak area (mAu*s)	Concentration recovery (mg/mL)	Recovery (%)
80%	8.0	0.8	3762027	0.787	98.357
	8.0	0.8	3784160	0.791	98.936
	8.0	0.8	3784160	0.791	98.936
100%	10.0	1.0	4698839	0.998	99.799
	10.0	1.0	4693053	0.997	99.676
	10.0	1.0	4693365	1.009	99.682
120%	12.0	1.2	5570250	1.188	99.978
	12.0	1.2	5570550	1.189	99.083
	12.0	1.2	5569939	1.188	98.978
Average recovery rate (%)					99.269
%RSD					0.539

[Data source: Experimental data generated by the authors using HPLC system LC-2030 3D; statistical parameters calculated using Microsoft Excel]

kinetics. The tailored HPLC method developed in this study overcomes these limitations by enabling accurate quantification of oxaliplatin in ZIF-8 formulations.

In contrast, UV-Vis spectroscopy, which is often used for quantifying drug loading in nanomaterials, has several limitations. UV-Vis typically lacks the specificity to distinguish oxaliplatin from other components, leading to less precise quantification. Moreover, its non-specific nature increases the likelihood of interference from other substances, compromising the reliability of drug content determination. The customized HPLC method, however, is specifically designed to account for oxaliplatin's unique characteristics within the ZIF-8 structure, making it a more reliable tool for assessing drug loading and release profiles compared to previous techniques.

With a broad linear range of 0.1 to 1.5 mg/mL and minimal RSD values, this method ensures accurate quantification across typical concentrations used in drug loading and release studies. Its versatility and robustness make it suitable for various stages of drug delivery system development, from initial loading experiments to in-depth studies on release kinetics.

In adherence to ICH guidelines, a comprehensive validation process has confirmed key criteria such as system suitability, specificity, linearity, accuracy, precision, range, and robustness, ensuring the method's reliability and applicability in drug delivery research. This thorough validation distinguishes the current

method from earlier reports, which may not have fully evaluated these critical analytical parameters.

The use of common solvents, reagents, and standard equipment enhances the method's accessibility across various laboratory settings. This analytical tool enables rapid, cost-effective analysis and supports more accurate comparisons, ultimately facilitating the advancement of innovative drug delivery systems utilizing ZIF-8.

CONCLUSIONS

This study has developed and validated an HPLC-PDA method for the accurate quantification of oxaliplatin incorporated into ZIF-8 structures. This approach allows for rapid and cost-effective analysis, effectively determining both drug incorporation and release rates. By providing a reliable analytical tool for complex ZIF-8 compositions, this methodology ensures the safety and efficacy of innovative drug delivery systems while establishing a foundation for accelerating future developments. Consequently, it is particularly beneficial for advancing research in this field in Vietnam.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Quyen Toan Pham: Methodology, Writing - review & editing, Validation, Visualization, Project admin-

istration, Resources. **Xuan Hiep Nguyen:** Methodology, Validation, Formal analysis, Writing - original draft, Resources. **Nhat Hoang Pham:** Investigation, Formal analysis, Methodology. **Linh Ho Thuy Nguyen:** Conceptualization, Methodology, Writing - review & editing, Visualization. **Minh Tri Le:** Conceptualization, Methodology, Writing - review & editing, Visualization. **Tan Le Hoang Doan:** Conceptualization, Methodology, Writing - review & editing, Visualization, Project administration, Resources.

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Xây dựng và thẩm định phương pháp RP-HPLC định lượng oxaliplatin trong hệ dẫn truyền thuốc trên cơ sở vật liệu ZIF-8

Phạm Toàn Quyền^{1,2,3}, Nguyễn Xuân Hiệp^{1,2}, Phạm Nhật Hoàng^{1,2}, Nguyễn Hồ Thùy Linh^{1,2}, Lê Minh Trí^{1,2,3}, Đoàn Lê Hoàng Tân^{1,4,*}



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¹Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

²Trường Đại học Khoa học sức khỏe, Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

³Khoa Dược, Đại học Y Dược Thành phố Hồ Chí Minh, Việt Nam

⁴Trung tâm Vật liệu Cấu trúc Nano và Phân tử (INOMAR), Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

Liên hệ

Đoàn Lê Hoàng Tân, Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

Trung tâm Vật liệu Cấu trúc Nano và Phân tử (INOMAR), Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

Email: dlhtan@inomar.edu.vn

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TÓM TẮT

Đặt vấn đề: Thuốc oxaliplatin được sử dụng rộng rãi trong điều trị ung thư nhưng thường bị hạn chế bởi các tác dụng phụ nghiêm trọng. Zeolitic Imidazolate Framework-8 (ZIF-8), một loại vật liệu cấu trúc khung hữu cơ kim loại có kích thước hạt nano, đã đang được nghiên cứu như một hệ vận chuyển thuốc tiềm năng cho oxaliplatin, nhờ những ưu điểm như khả năng hướng đích tế bào ung thư và giải phóng thuốc có kiểm soát. Nghiên cứu này nhằm xây dựng và thẩm định phương pháp sắc ký lỏng hiệu năng cao pha đảo (RP-HPLC) với độ nhạy, tính chính xác cao nhưng đơn giản, hiệu quả và kinh tế để định lượng oxaliplatin tải trên vật liệu ZIF-8. **Phương pháp:** Nhiều điều kiện sắc ký khác nhau đã được khảo sát để định lượng chính xác oxaliplatin nạp vào ZIF-8. Phương pháp HPLC cuối cùng được thẩm định theo hướng dẫn của Hội đồng hài hòa hóa các yêu cầu kỹ thuật đối với dược phẩm dùng cho người (ICH). **Kết quả:** Việc tách sắc ký được thực hiện trên cột RP-C18 (250 x 4,6 mm, 5 μ m) bằng chế độ rửa giải đẳng dòng với pha động methanol-nước (tỷ lệ 1:1), tốc độ dòng 0,8 mL/phút. Tín hiệu được ghi nhận bằng đầu dò PDA tại bước sóng 230 nm. Phương pháp đã được chứng minh tính phù hợp thông qua thẩm định các chỉ tiêu: tính đặc hiệu, tính tuyến tính, độ chính xác, độ đúng, khoảng xác định và độ thô. Tính tuyến tính được xác nhận trong khoảng nồng độ 0,1-1,5 mg/mL, với hệ số tương quan (R^2) đạt 0,9994. Độ chính xác nằm trong khoảng 98,79% - 99,71% (trung bình 99,16%) và độ lệch chuẩn tương đối (RSD) là 0,48%. **Kết luận:** Phương pháp RP-HPLC đã thẩm định là công cụ phân tích đáng tin cậy để xác định lượng thuốc tải cũng như hiệu suất giải phóng oxaliplatin của ZIF-8. Kết quả này không chỉ đóng vai trò thiết yếu trong việc đánh giá hệ vận chuyển thuốc mà còn hỗ trợ kiểm soát chính xác đặc tính hệ vận chuyển thuốc, góp phần thúc đẩy sự phát triển của các ứng dụng trong nghiên cứu hệ vận chuyển thuốc tương lai.

Từ khoá: Oxaliplatin, HPLC, định lượng, vật liệu ZIF-8, hệ vận chuyển thuốc

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