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Research Article

Developing and validation of high performance liquid chromatography method for determination of carboplatin-loaded Metal-organic framework materials

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ABSTRACT

Introduction: Carboplatin is widely used in cancer treatments despite several adverse effects. Metal-organic framework nanoparticles (MOF) were used to load carboplatin, which could improve selectivity to cancerous tissues and control the release profile of this compound. This study aimed to develop and validate a sensitive, simple, cost-effective RP-HPLC method for quantifying carboplatin in MOF.

Methods: This study was developed and optimal different chromatographic conditions for the quantification of carboplatin-loaded MOF materials. The chosen HPLC procedure was validated throughout ICH guidelines.

Results: The chromatographic separation was achieved on an RP-C18 column (250 x 4.6 mm, 5 μm) by isocartic elution with a mobile phase consisting of methanol: potassium chloride 0.9% in a 1:1 ratio. The flow rate is 1.0 mL/min. PDA detector was used at a wavelength of 254 nm. The validation process has achieved the following criteria: system suitability, specificity, linearity, accuracy, precision, range of determination, and robustness. The method showed linearity in the 0.1-1.5 mg/mL range with $R^2 = 0.9998$. The accuracy and %RSD were 98.03-101.37% (mean 99.98%), and 1.08, respectively.

Conclusion: This HPLC method can be used to determine the carboplatin loading and desorption efficiency of MOF materials, including ZIF-8, Zr-UiO-66, and Hf-UiO-66. The method provides a reliable analytical tool for complex MOF compositions, ensuring the safety and efficacy of novel drug delivery systems while accelerating future development in this field.

Key words: Carboplatin, HPLC, quantitative evaluation, MOF materials, drug delivery system

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INTRODUCTION

Cancer is one of the leading causes of death worldwide, which poses a significant burden on the economies of many countries [1]. The treatment of cancer often relies on chemotherapy, where drugs are used to kill cancer cells, prevent growth, and inhibit metastasis to other organs [2]. Platinum-based drugs, particularly carboplatin, are widely used in cancer therapy. However, these active ingredients often require high doses yet exhibit low selectivity, leading to reduced treatment efficacy and increased adverse effects [3]. Recent research efforts have focused on the application of metal-organic framework (MOF) nanoparticles as drug delivery systems to overcome these limitations. MOFs have shown promise in loading and delivering chemotherapeutic agents such as carboplatin, with the potential to protect the active pharmaceutical ingredient from decomposition, enhance bioavailability, and provide controlled release profiles [4 5 6]. Previous studies have primarily relied on Inductively Coupled Plasma (ICP) measurements to quantify drug loading onto nanomaterials, which lack the specificity and sensitivity offered by High Performance Liquid Chromatography (HPLC). Furthermore, existing HPLC methods for quantifying free carboplatin or carboplatin in injectable formulations cannot be directly applied to accurately determine the drug loading capacity and release efficiency in these novel delivery systems, due to the distinct characteristics of carboplatin-loaded MOFs compared to unmodified carboplatin. Consequently, there is a need to establish a quantitative method for determining the API in carboplatin-loaded MOF materials and their release profiles, which has a simple procedure, is easy to prepare, and has high accuracy and reliability [7]. The objective of this study is to develop and validate an HPLC method for determining carboplatin in mixtures with MOFs, evaluating the loading and desorption efficiency of the corresponding metal-organic-

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framework materials – an approach not previously reported in the literature.

METHODS

Materials

Chemical reagents and solutions: Carboplatin material range was kindly provided by Bidiphar (Vietnam) (Lot. No. CA-22060) as a gift and carboplatin standard BP was purchased from Merck (Germany) (Lot. No. R152G0). These MOF materials consisting of Zeolitic Imidazolate Framework-8 (ZIF-8), Zirconium-based University of Oslo-66 (Zr-UiO-66), and Hafnium-based University of Oslo-66 (Hf-UiO-66) were synthesized according to the published procedure of INOMAR. Potassium chloride in analytical grade from Fisher (USA), methanol, and water for HPLC were supplied by Merk (Germany).

Instruments: A Shimadzu HPLC system LC-2030 3D with a Photo Diode Array (PDA) detector (Japan) was used. Data processing was performed with Lab Solution software (Japan). A Phenonemex Reverse Phase C18 (RP-C18) column (250 x 4.6 mm, 5 μm) (USA) and other precision glassware of analytical grades were used for this study.

Preparation of solutions

Stock solution: Exactly 20.0 mg carboplatin material range was dissolved by 0.9% KCl in a 20 Ml of volumetric flask and then diluted up to the mark.

Carboplatin@MOF sample solutions: Weigh exactly 5.0 mg of each complex material into amber colored flask, and 5 mL stock solution was added. The mixture was stirred for 24 hours. After centrifuging the mixture, the supernatant was collected and filtered through a 0.45 μm nylon filter into a 1.5 mL vial.

Standard solution: Exactly 20.0 mg carboplatin standard was dissolved by 0.9% KCl in a 20 mL volumetric flask and then diluted up to the mark. The solution was filtered through a 0.45 μm nylon filter into a 1.5 mL vial.

HPLC conditions optimization

Determination of detection wavelength: The UV-Vis spectrum in HPLC chromatography was used to determine the maximum absorbance wavelength for carboplatin.

Optimization of method: different ratios of mobile phases were tested with an RP-HPLC column and PDA detector. The suitable chromatographic conditions were selected based on the retention time, resolution, tailing factors, and peak purity.

Validation of analytical method

The developed HPLC with suitable conditions was validated according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R1) guidelines for analytical parameters[8] such as system suitability, specificity, linearity, accuracy, precision, range, limit of detection (LOD), limit of quantitation (LOD), and robustness.

RESULTS

HPLC conditions optimization

Chromatographic separations were carried out using the Phenomenex RP-C18 column (250 x 4.6 mm; 5 μm) at room temperature with isocartic mode using a mobile phase of methanol and 0.9% KCl in a ratio of 1:1. The flow rate was 1.0 mL/min with the injection volume was 20 μL. All chromatographs were measurements at detection wavelength 254 nm. The result chromatographic showed carboplatin's retention time (t_R) was 2.9 min and had good peak parameters (Figure 1).

Validation of analytical method

System suitability: A standard solution was injected and repeated 6 times to record results. Table 1 showed that % Relative Standard Deviation (RSD) of these parameters meets the acceptance required not more than 2.0%. Hence, the method was archived for the system suitability.

Specificity

To confirm the capacity of the analytical method to measure the presence of identified compound separately and specifically from other components in the mixture. In this study, specificity was evaluated for carboplatin, MOF material individually, and the combinations in both normal and stress conditions (stay exposed to light for 24 hours at room temperature). Furthermore, peaks corresponding were also evaluated on peak purity.

Figures 2 to 12 showed a chromatographic of the specificity study, which illustrated that carboplatin completely separated and separated of other components the mixture. The standard solution had only one peak of carboplatin at 2.9 min. In contrast, the blank chromatographic using the diluent did not show any peak. Moreover, the chromatography of each MOF material sample had other peaks but those retention times were different from carboplatin's peak. In the added-standard samples, the carboplatin's peak area and height were increased more than in the basic

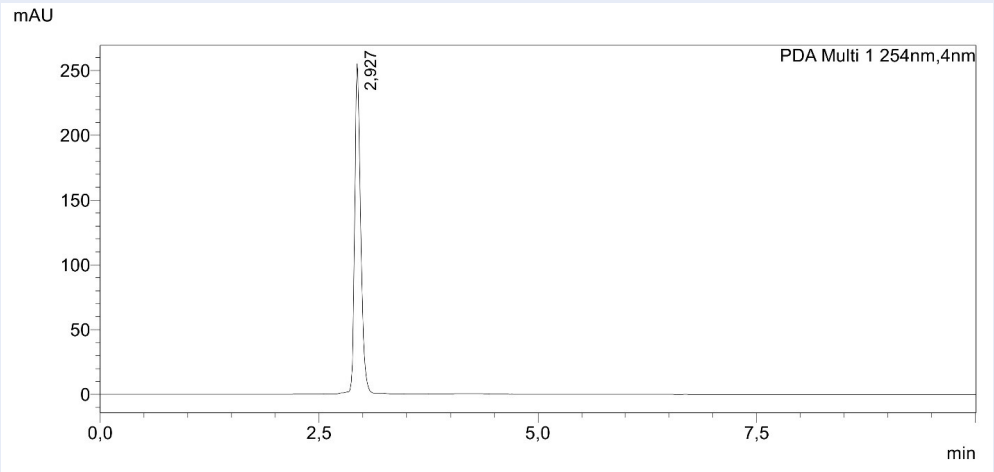

Figure 1: Chromatography of carboplatin standard

Table 1: Results of system suitability (n=6)

S.No	tR (min)	Area (mAU*s)	Tailing factor	Resolution	Theoretical plate count
1	2.932	1283963	1.385	1.629	6461
2	2.929	1281430	1.389	1.719	6466
3	2.930	1280609	1.394	1.809	6420
4	2.930	1280528	1.423	1.901	6533
5	2.933	1283066	1.417	1.953	6340
6	2.932	1269221	1.420	1.966	6446
Mean	2.931	1279802.833	1.405	1.8295	6444
Accepteance criteria	-	-	0.8 – 1.5	≥ 1.5	> 2000
Standard deviation	0.002	5361.975	17.1425	7.408	63.387
%RSD	0.0529	0.419	1.220	0.018	0.983

sample, whenever other peaks' parameters were not changed.

Besides, the stress condition sample for 24 hours illustrated new purity peaks, which also separated clearly from carboplatin's peak (Figures 13 to 15). The result in peak purity met the required more than 99.99%. Based on this information, the analytical method was confirmed specificity parameter.

Linearity

To evaluate the linearity of carboplatin in this method, several different concentrations of standard solutions were prepared concluding with 0.1 – 1.5 mg/mL of carboplatin content (Table 2). Each concentration was triple injections under the same conditions. The mean of peak areas were used to analyze the calibration curve based on the least square linear regression method.

The linear equation of carboplatin was obtained $\hat{y}$= 1282701.83x + 30208.36, and the goodness-of-fit (R^2) was found to be 0.9998 (Figure 16), indicating that there is a linear relationship between the concentration of carboplatin and peak area. Hence, the HPLC method met the requirement of linearity in the range of 0.1 to 1.5 mg/mL of carboplatin content.

Precision

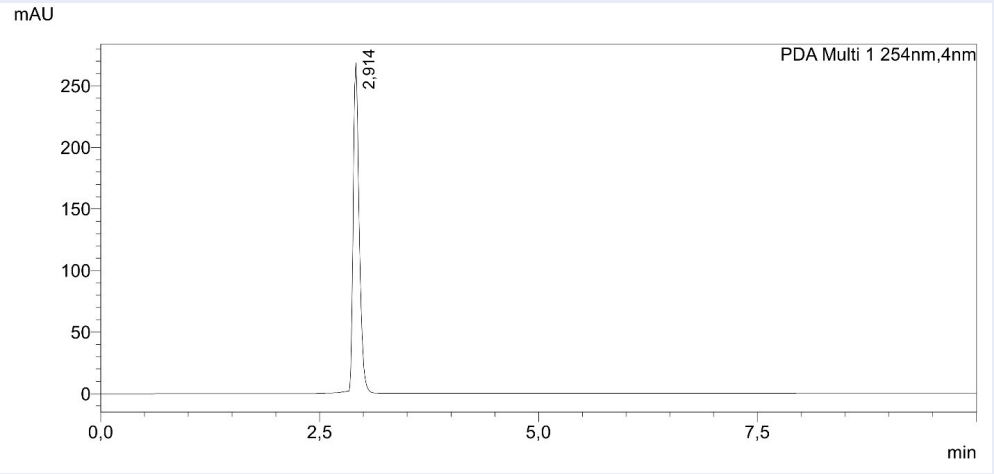

Figure 2: Chromatograms of the specificity resultingfrom standard solution

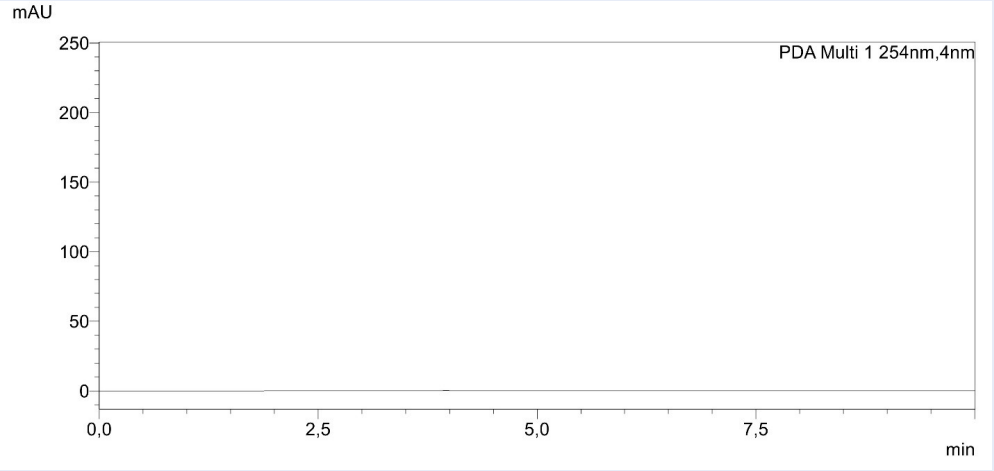

Figure 3: Chromatograms of the specificity resultingfrom blank solution

Table 2: The results of linearity

Concentration (mg/mL)	Peak area (mAu*s)			Mean (mAu*s)	%RSD
0.1	153253	153262	152895	153136.7	0.137
0.2	288459	288548	289701	288902.7	0.240
0.4	531300	529775	528350	529808.3	0.278
0.5	681842	681722	681234	681599.3	0.047
0.8	1067879	1067839	1067854	1067857	0.002
1.0	1317498	1317331	1315930	1316920	0.065
1.2	1568510	1568153	1565900	1567521	0.090
1.5	1947229	1946988	1947748	1947322	0.019

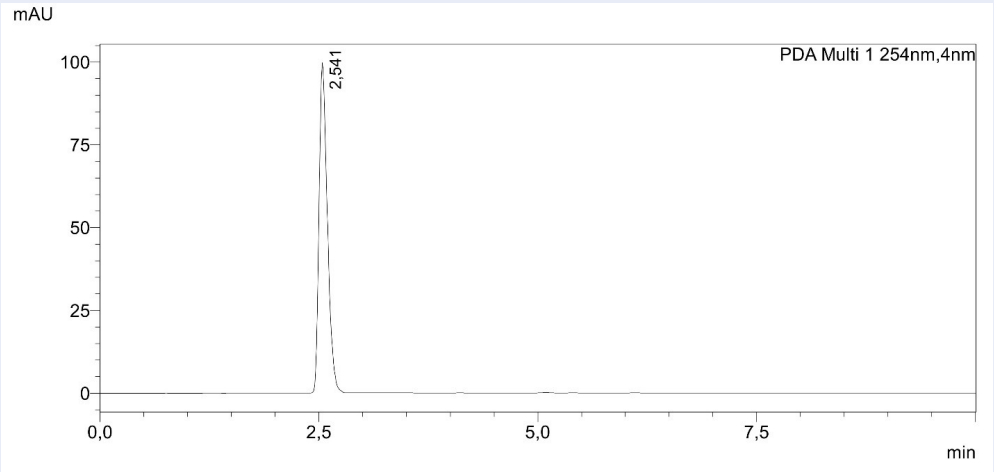

Figure 4: Chromatograms of the specificity resultingfrom Hf-UiO-66 material

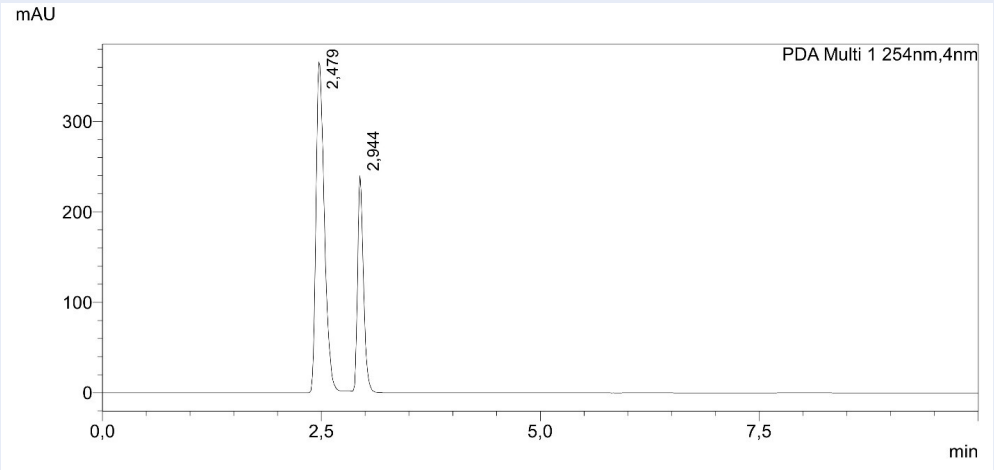

Figure 5: Chromatograms of the specificity resultingfrom CBP@Hf-UiO-66 sample

The precision parameter included repeatability and intermediate precision by several measurements of standard solution. The repeatability was established by six standard solutions at the 100% concentration levels on the same day. The intermediate precision was established by twelve standard solutions at 100% concentration levels, which were prepared by two members on two different days. The RSD of carboplatin content was calculated based on the peak area results.

The precision results were shown in Tables 3 and 4, which indicated the analysis method met the requirements with RSD values (0.103 and 0.260, respectively) of no more than 2%. Using F-test and t-test to statistic the data analysis, the establishment method showed significant differences between two members on variance but were not significant differences on average, and $T_{tn} < T_{0.05}$. Hence, the HPLC procedure archived the precision parameter.

Accuracy

To evaluate the accuracy of the method, three standard solutions were prepared by three members at three different times at three concentration levels concluding 50%, 100%, and 150%. Each concentration sample was injected triple. The percentage recovery of added carboplatin and RSD of recovery rate was calculated for those results (Table 5).

The accuracy results showed percentage recovery and RSD values of all solutions at three levels in the range of 98.03-101.37% and 1.08%, respectively. That indi-

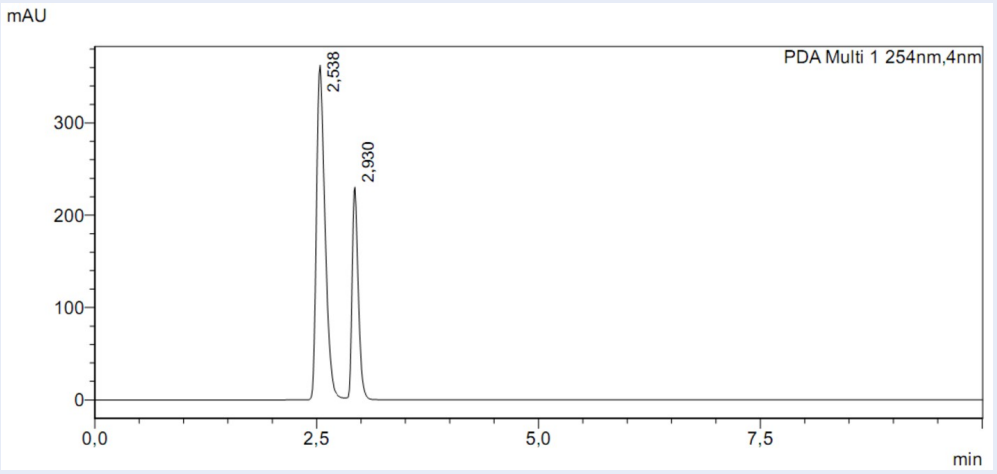

Figure 6: Chromatograms of the specificity resultingfrom CBP@Hf-UiO-66 standard

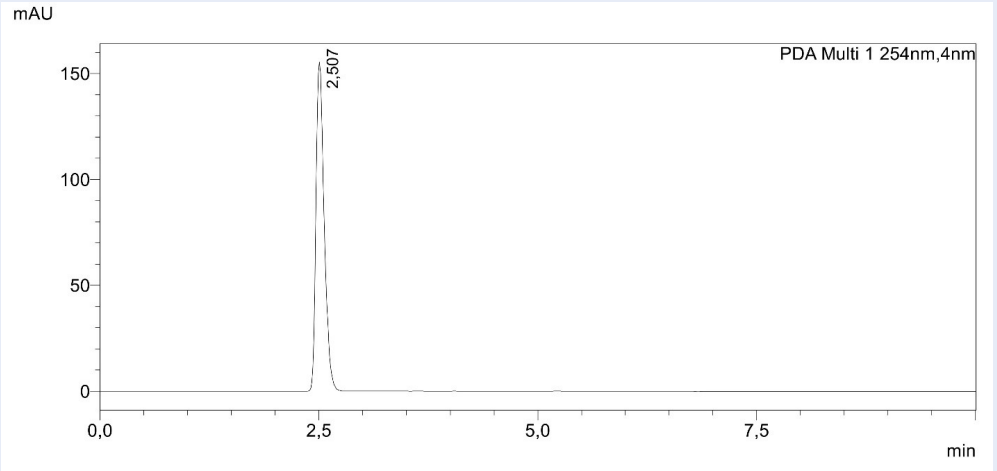

Figure 7: Chromatograms of the specificity resultingfrom Zr-UiO-66 material

Table 3: Results of repeatability

Sample No.	Weight (mg)	Peak area (mAu*s)	Content (%)
1	19.9	1315534	99.895
2	20.0	1317498	100.044
3	20.0	1317331	100.031
4	19.9	1315930	99.925
5	20.0	1317736	100.062
6	20.1	1319314	100.182
	Mean (%)		100.023
	%RSD (n =6)		0.103

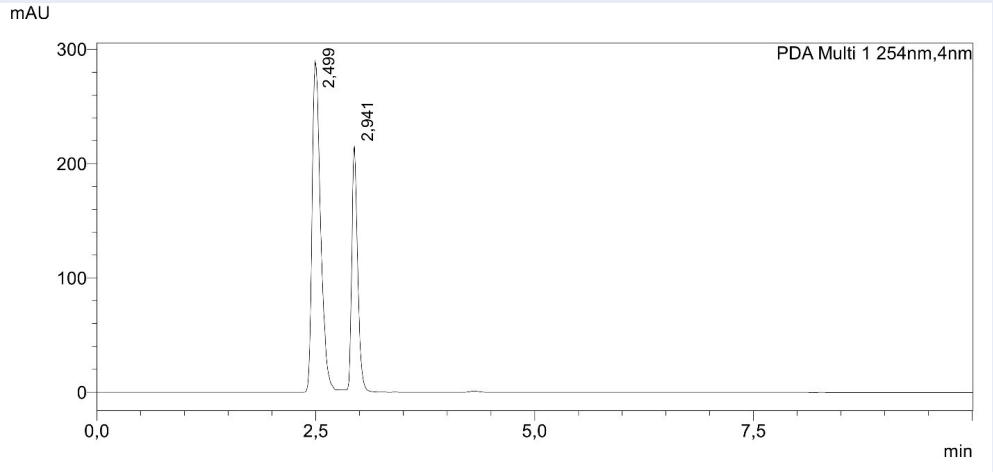

Figure 8: Chromatograms of the specificity resultingfrom CBP@Zr-UiO-66 sample

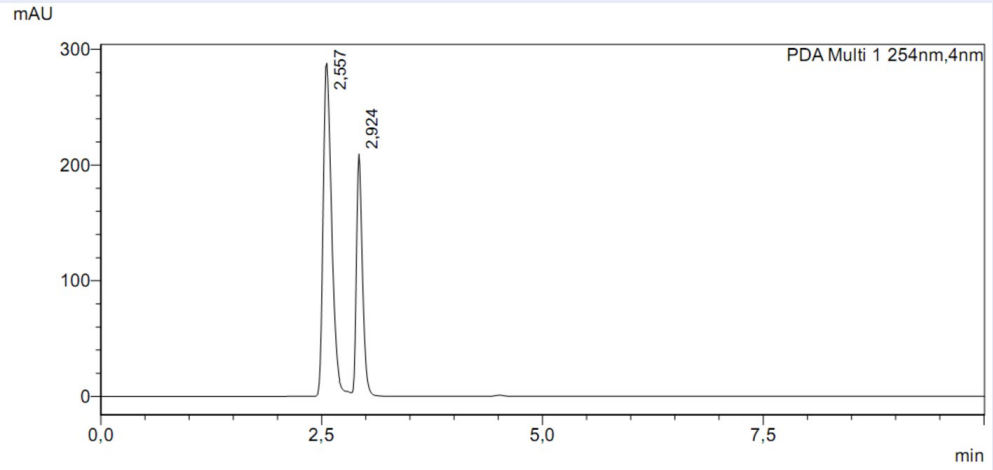

Figure 9: Chromatograms of the specificity resultingfrom CBP@Zr-UiO-66 standard

cates the ability to apply the method to determine carboplatin in the mixture with MOF materials.

Range: Based on these parameters concluding of linearity, precision, and accuracy, the range of the method was evaluated by 0.4 – 1.2 mg/mL.

Robustness: Several minor changes in the experimental parameters were analyzed to be evaluated by calculated RSD of parameters: flow rate: ± 0.1 mL/min; column temperature: ± 2 ^{o}C; different vial numbers in the tray. The results of chromatography indicated the procedure met the requirements of %RSD, tailing factor, and resolution.

DISCUSSION

This analytical technique, which employs reverse-phase chromatography with a PDA detector and an ODS-C18 column, was developed based on the polarization characteristics of carboplatin. The optimized mobile phase (a 1:1 mixture of methanol and 0.9% KCl) enhances the stability of the target compound while facilitating its complete isolation from other substances at 254 nm.

The procedure's efficacy is evident in its ability to isolate carboplatin from diverse components, even under stress conditions, which is crucial for the precise quantification of drug loading and release kinetics in MOF-based delivery systems. Its high sensitivity and specificity are particularly valuable when analyzing complex matrices like MOFs, where potential interference from metallic ions or other elements could impact the precision of the results. This effectiveness has been demonstrated through its application

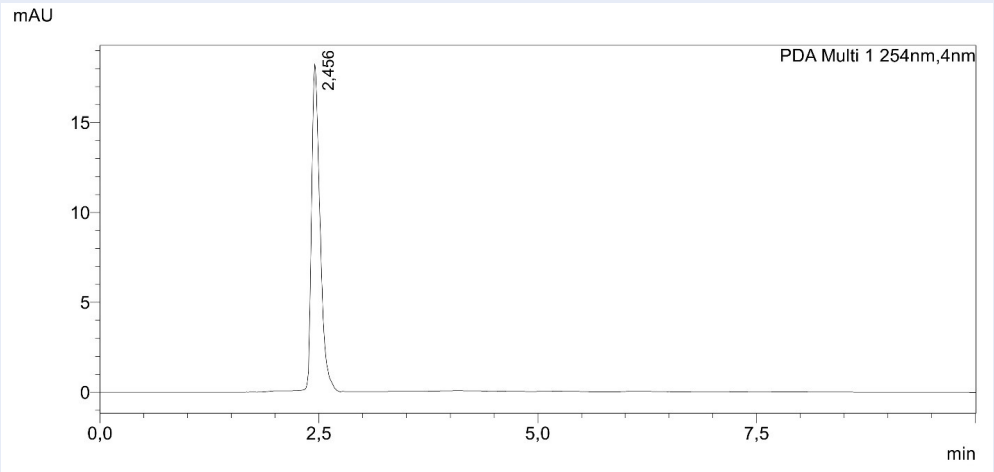

Figure 10: Chromatograms of the specificity resultingfrom ZIF-8 material

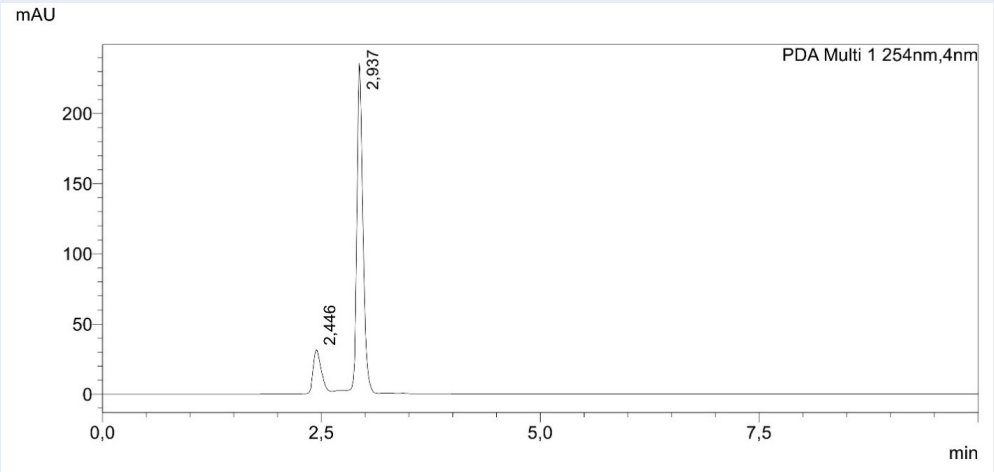

Figure 11: Chromatograms of the specificity resultingfrom CBP@ZIF-8 sample

to three different MOF materials (ZIF-8, Zr-UiO-66, and Hf-UiO-66), showcasing its potential for broader use in optimizing drug delivery systems.

Compared to previous studies that primarily relied on Inductively Coupled Plasma (ICP) measurements to quantify carboplatin loading onto nanomaterials[9], this HPLC method offers superior specificity and sensitivity for accurately determining the drug content in carboplatin-loaded MOF systems. Unlike generic HPLC methods for free carboplatin or carboplatin in injectable formulations, this technique is specifically designed to account for the unique characteristics of carboplatin-loaded MOFs, enabling more accurate quantification of drug loading capacity and release profiles.

In contrast, the use of UV-Vis spectroscopy for quantifying drug loading onto nanomaterials has several drawbacks. UV-Vis measurements often lack the specificity to distinguish carboplatin from other components in the complex nanomaterial matrix, leading to less accurate quantification[10]. Furthermore, the non-specific nature of UV-Vis makes it more prone to interference from other substances present in the nanomaterial samples, compromising the reliability of drug content determination. Unlike the tailored HPLC technique, generic UV-Vis methods cannot adequately account for the specific behaviors of carboplatin when incorporated into MOF structures. Therefore, the HPLC approach demonstrated in this work offers a more reliable analytical tool for evaluating drug-loading capacities and release profiles of

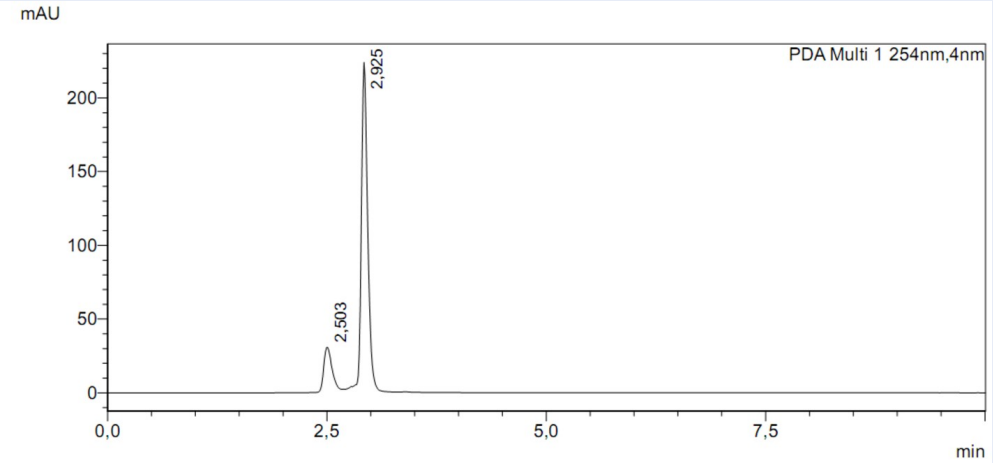

Figure 12: Chromatograms of the specificity resultingfrom CBP@ZIF-8 standard

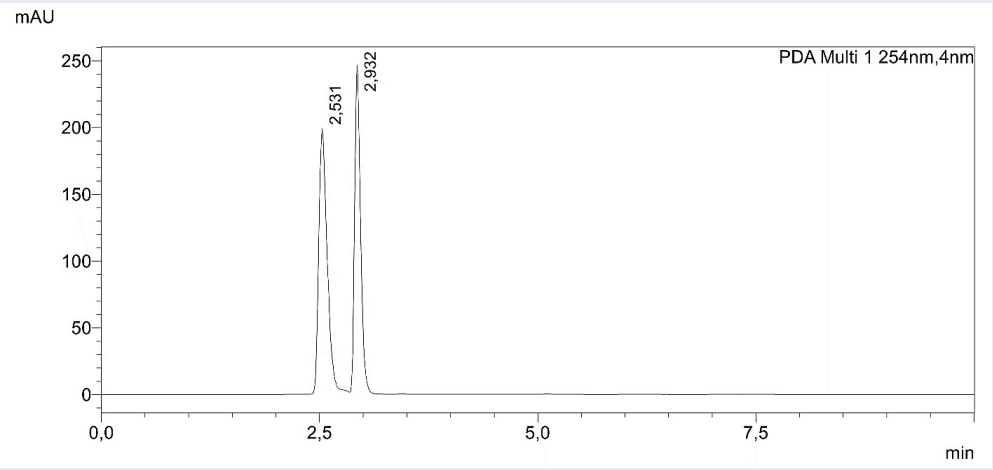

Figure 13: Chromatograms of the specificity resulting from CBP@Hf-UiO-66 in stresscondition

carboplatin-loaded MOF materials compared to previous techniques.

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

Adhering to ICH guidelines, the rigorous evaluation process has validated key parameters, including system suitability, specificity, linearity, accuracy, precision, range, and robustness, ensuring reliability and applicability in drug delivery system investigations. This comprehensive validation distinguishes the current method from previous reports, which may have lacked such a thorough evaluation of the analytical technique.

The utilization of common solvents, reagents, and equipment enhances the technique's accessibility across diverse laboratory settings. This analytical tool enables rapid, cost-effective analysis and facilitates more accurate comparisons between various MOF materials and formulations, ultimately accelerating the development of innovative drug delivery systems.

CONCLUSIONS

This research has established and validated an HPLC-PDA technique for precise quantification of carboplatin incorporated into MOF structures. The approach enables swift, economical analysis, accurately

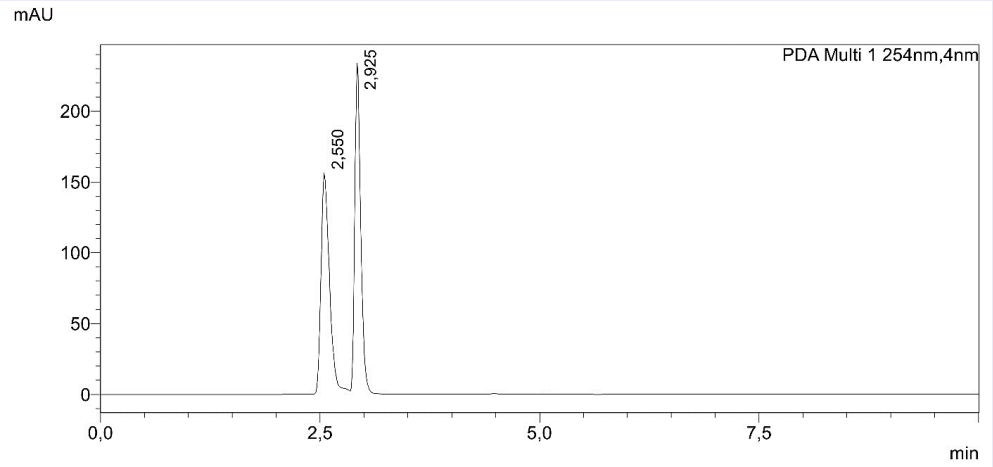

Figure 14: Chromatograms of the specificity resulting from CBP@Zr-UiO-66 in stresscondition

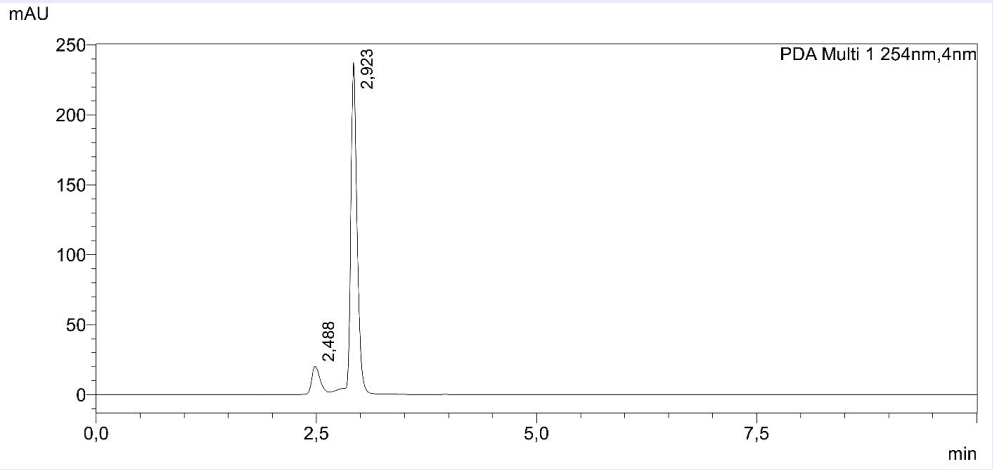

Figure 15: Chromatograms of the specificity resulting from CBP@ZIF-8 in stresscondition

determining both incorporation and release rates. By providing a reliable analytical tool for complex MOF compositions, this methodology not only ensures the safety and efficacy of novel drug delivery systems but also lays the groundwork for accelerating future development. Consequently, it proves especially valuable in advancing research within 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 administration, Resources. **Hoang-Han Do:** Methodology, Validation, Formal analysis, Writing - original draft, Resources. **Truc Thanh Nguyen:** Investigation, Formal analysis, Writing - original draft. **Huong Thi Thanh Pham:** Validation, Resources, Visualization. **Linh Ho Thuy Nguyen:** Conceptualization, Methodology, Writing - review & editing, Visualization. **Tan Le Hoang Doan:** Conceptualization, Methodology, Writing - review & editing, Visualization. **Tri Minh Le:** Conceptualization, Methodology, Writing - review & editing, Visualization, Project administration.

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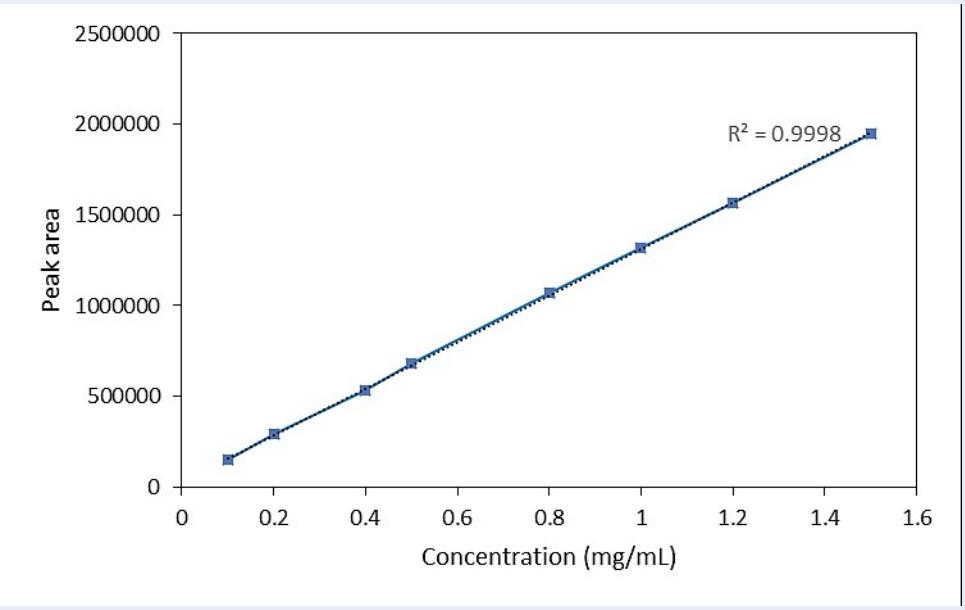

Figure 16: Regression correlation curve of carboplatin

Table 4: Results of intermediate precision

Sample	Member 1			Member 2		
	Weight (mg)	Peak area (mAu*s)	Content (%)	Weight (mg)	Peak area (mAu*s)	Content (%)
1	19.8	1304370	99.047	19.7	1299603	98.685
2	19.8	1304858	99.084	19.8	1300437	98.748
3	19.9	1305483	99.132	19.8	1301431	98.824
4	19.9	1306521	99.210	19.8	1302895	98.935
5	19.9	1306202	99.186	19.8	1303316	98.967
6	19.7	1292943	98.179	19.8	1303282	98.964
	Mean (%)		98.973	Mean (%)		98.854
	%RSD (n =6)		0.122	%RSD (n =6)		0.398
	Average content (%) of two members (n = 12): 98.914 %RSD (n=12): 0.260					
Ttn < T0.05 = 2.447					0.505	

number C2022-44-05.

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Table 5: Results of accuracy

Level (%)	Weight added (mg)	Concentration added (mg/mL)	Mean of peak area (mAu*s)	Concentration recovery (mg/mL)	Recovery (%)
80%	15.3	0.8	1014750.333	0.811	101.369
	15.2	0.8	1014282	0.799	99.892
	15.4	0.8	1026602	0.802	100.270
100%	19.1	1.0	1251560	1.000	100.020
	19.4	1.0	1277511	1.001	100.663
	19.6	1.0	1291088	1.009	100.882
120%	23.3	1.2	1504157	1.202	100.200
	23.2	1.2	1500484	1.182	98.517
	23.5	1.2	1505549	1.176	98.033
Average recovery rate (%)					99.983
%RSD					1.077

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Bài nghiên cứu

Xây dựng và thẩm định quy trình định lượng vật liệu nano khung hữu cơ-kim loại (MOF) tải carboplatin

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TÓM TẮT

Giới thiệu: Carboplatin được sử dụng rộng rãi trong điều trị ung thư nhưng lại có nhiều tác dụng phụ. Vật liệu khung hữu cơ-kim loại (MOF) được ứng dụng để làm chất mang nano tải carboplatin, giúp tăng khả năng chọn lọc đối với mô ung thư và kiểm soát quá trình giải phóng thuốc nhằm giảm thiểu tác dụng phụ. Nghiên cứu này nhằm phát triển và thẩm định một quy trình RP-HPLC có độ nhạy, tính chính xác, đơn giản và tiết kiệm chi phí để định lượng hoạt chất carboplatin tải lên vật liệu MOF. **Phương pháp:** Nghiên cứu này đã xây dựng và phát triển các điều kiện sắc ký khác nhau để định lượng vật liệu nano MOF tải carboplatin. Phương pháp HPLC lựa chọn được tiến hành thẩm định các chỉ tiêu theo hướng dẫn của ICH. **Kết quả:** Sự tách sắc ký được thực hiện trên cột RP-C18 (250 × 4,6 mm, 5 μm) bằng phương pháp rửa giải đẳng dòng với pha động gồm methanol và kali clorid 0,9% theo tỷ lệ 1:1. Tốc độ dòng là 1,0 mL/phút. Đầu dò PDA được sử dụng ở bước sóng 254 nm. Quá trình thẩm định đã đạt đầy đủ các chỉ tiêu: độ phù hợp hệ thống, độ đặc hiệu, tính tuyến tính, độ chính xác, độ lặp lại, khoảng xác định và độ bền vững. Phương pháp cho thấy tính tuyến tính trong khoảng 0,1–1,5 mg/mL với hệ số tương quan R^2 = 0,9998. Độ chính xác và %RSD lần lượt là 98,03–101,37% (trung bình 99,98%) và 1,08. **Kết luận:** Phương pháp HPLC có thể được sử dụng để xác định hàm lượng và hiệu suất giải phóng carboplatin từ các vật liệu MOF, bao gồm ZIF-8, Zr-UiO-66 và Hf-UiO-66. Kỹ thuật này cung cấp một công cụ phân tích đáng tin cậy cho các thành phần MOF phức tạp, đảm bảo tính an toàn và hiệu quả của các hệ phân phối thuốc mới, đồng thời thúc đẩy sự phát triển nghiên cứu trong lĩnh vực này.

Từ khoá: Carboplatin, HPLC, đánh giá định lượng, vật liệu nano MOF, hệ phân phối thuốc

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