DEVELOPMENT AND VALIDATION OF QUANTITATIVE ESTIMATION OF POMALIDOMIDE IN CAPSULE DOSAGE FORM BY RP-HPLC

Amjad Ali M.Iqbal[1*], Ashwini Patil[2], Sayali S. Gaikwad[3], Shakeel .A. Choudhary[4], Kaleem Mazhar Petkar[5]

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Abstract:

Pomalidomide is a derivative of Thalidomide and marketed by Celgene and can be enclosed it under the class of medicines called as an immune-modulatory agents. Aim of this research article is to produce a gradient elution method of analysis which is simple, linear, precise, rapid and accurate for reverse phase High Performance Liquid Chromatographic method for the quantitative estimation of Pomalidomide drug in Hard Capsule dosage form using chromatographic conditions like, Kinetex Phenyl Hexyl XB-C18, (250x4.6mm, 5µm particle size) column with mobile phase consisting of Solvent-A Phosphate Buffer and Solvent-B as Methanol in gradient mode of elution was used keeping flow rate 1.0 ml/min and wavelength at 225 nm. The retention time was found to be 09-13 min for the run time 25 minutes. The method was validated by determining it's linearity, accuracy, precision (System precision and Method precision), robustness. The detector response was linear for the concentration range of 24-36 mcg/ml. The linear regression equation found to be Y =77701X+7076.6. Most importantly results of the study proved that the proposed RP-HPLC method is useful for the routine determination of Pomalidomide in bulk drug and pharmaceutical Hard gelatin shell Capsule dosage form.

keywords: Pomalidomide; RP-HPLC; Hard Shell Capsules; Gradient Flow,ICH Guideline.

[1*]Department of Pharmaceutical Chemistry & Quality Assurance at Oriental College of Pharmacy, Maharashtra, India.
[2,3,4,5]Department of Quality Assurance at Oriental College of Pharmacy, Maharashtra,India.

***Corresponding Author**
Amjad Ali M.Iqbal[1*]
[1*]Department of Pharmaceutical Chemistry & Quality Assurance, Oriental College of Pharmacy, Mumbai University, Sanpada, Mumbai-400705, Maharashtra, India.
Email address: [1*]amjad.ali@ocp.edu.in

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1. INTRODUCTION

Pomalidomide is an orally bioavailable immunomodulating, antiangiogenic and antineoplastic activities as per the reported studies available for the same drug. Most of these studies have indicated that pomalidomide may be useful in patients with multiple myeloma that is not responding to other treatments. The drug, a capsule taken by mouth, belongs to a class of medications called immunomodulating agent. Pomalidomide helps in the treatment of few symptoms like, it helps the bone marrow to produce normal blood cells, appears to inhibit TNF-alpha production, and it enhances the ability of immune cells to kill abnormal cells or abnormally growing cells in the bone marrow i.e., enhances the activity of T cells and natural killer (NK) cells and enhance antibody-dependent cellular cytotoxicity (ADCC). In addition, pomalidomide inhibit tumor angiogenesis (Production of new blood vessel from pre-existing vessels), promote cell cycle arrest in susceptible tumor cell populations, and stimulate erythropoiesis (Production of Red Blood Cell).

The active substance is in crystalline form and non-hygroscopic in nature with yellow colour appearance, which is slightly soluble in acetone, acetonitrile, methylene chloride, methyl ethyl ketone and tetrahydrofuran; very slightly soluble in absolute ethanol, ethyl acetate, heptane, methanol, 2-propanol and toluene; and practically insoluble in water as per solubility studies reported till now. Pomalidomide have CAS (Chemical Abstracts Service) number as 19171-19-8 and molecular formula as C_{13}-H_{11}-N_3-O_4. Pomalidomide has one stereochemical center. The active substance is obtained as a racemic mixture with no any optical rotation were observed. Pomalidomide is just a structural analogue of lenalidomide and thalidomide and it cannot be derived from any of these compounds in a simple synthetic modification or in vivo. Therefore, pomalidomide is not considered to be a derivative of lenalidomide or thalidomide. Pomalidomide with dexamethasone is indicated in the treatment of patients with relapsed and refractory multiple myeloma who have received atleast two prior treatment regimens, including lenalidomide and bortezomib, and have demonstrated disease progress on the last therapy for adult patients.

The chemical name of pomalidomide is *(RS)*-4-Amino-2-(2,6-dioxopiperidin-3-yl)-isoindole-1,3-dione and has the following structure:

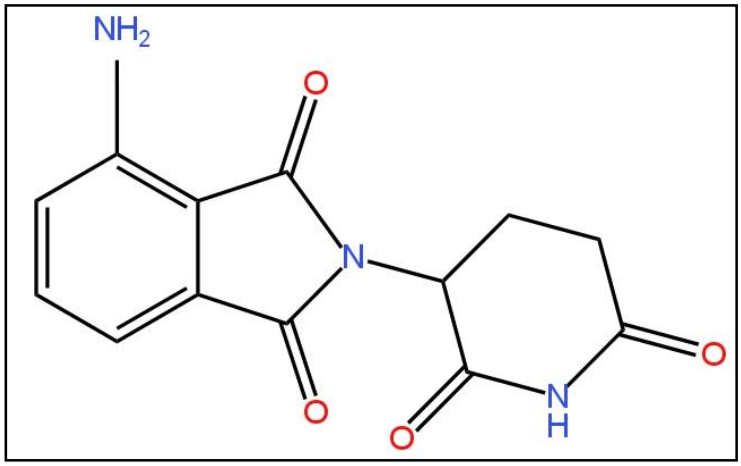

Figure 1: Chemical Structure of Pomalidomide

Several methods were reported for quantitative estimation of Pomalidomide and most of them were Isocratic elution method which can't consider as specific or sensitive towards the drug as compare to gradient method. To get sensitive or specific method towards drug one should develop a gradient elution method and for this only three methods were reported, one method is specific for tablet dosage form hence in this article, method is developed for the analysis of capsule dosage form. Another two methods were quite complex in terms of solution preparations like mobile phase, standard & sample solutions and requires much more quantity of the sample solution, hence this article will provide specifically sensitive method towards drug with lesser sample quantity requirement and less complex method for sample, standard, mobile phase and diluent preparation as compare to available method.[1-9]

2. RESULTS

Validation parameters

Developed RP-HPLC Quantitative method was validated by the different validation parameters as per ICH guidelines specifically ICH Q2(R1) guideline namely System suitability, Specificity,

Linearity, Accuracy, Precision (System precision and Method precision), Robustness.

2.1. System suitability:

System suitability testing is an integral part of most analytical procedures. The tests are based on the concept that system should work efficiently with the newly developed method so that if any issue is observed in the system due to any of the factor including instrumentation, preparation and any of the chromatographical condition can get resolved at this stage itself. System suitability test parameters was established for a type of procedure being validated which means parameters can differ from one procedure to the another. The system suitability tests were carried out on freshly prepared standard solution of Pomalidomide. Solution was injected six times in a row to observed the system suitability and the chromatograms were recorded. The system suitability parameters were evaluated from standard Chromatograms by considering the % RSD of areas, tailing factor, number of theoretical plates calculated by the system and fulfillment of acceptance criteria as per guidelines which are tailing factor should not be more than 2 units, theoretical plates should be more than 2000 and %RSD of peak areas should not be more than 2%. The tailing factors for Pomalidomide were 1.0 and USP theoretical plates were found to be significantly high around 8315 and %RSD for peak areas was found to be 0.96%, Hence the system was suitable for use.

Table No. 1: System suitability test of Pomalidomide

SR. No.	Area	Theoretical plates	Tailing factor
1	2308365	8586	1.0
2	2259221	7985	0.9
3	2284630	8500	1.0
4	2314801	8240	1.0
5	2290149	9021	1.0
6	2267122	7559	0.9
Mean	2287381	8315	1.0
Standard Deviation	21958.27		
%RSD*	0.96		

* Relative Standard Deviation

2.2. Specificity

Specificity is the ability of a system to measure accurately and specifically the analyte of interest in thepresence of other components which can be other excipients or impurities which could be process related or any other kind of impurities that may be expected to be present in the sample matrix. Thetermsselectivityandspecificity of the method or system areused ofteninterchangeably. Itis themeasure of the degree of interferencefrom otheractiveingredients,excipients,impuritiesanddegradationproducts,ensuringthatapeakresponseisduetoa singlecomponentonly Specificity is one of the validation parameters in which the blank, placebo, control, sample solutions are prepared as per the developed method and injected into thesystemsoastocheckforanyinterferenceattheretentiontimeofPomalidomide. There are no any peaks were observed in the blank and placebo which have an interference at the retention time of Pomalidomide hence, developed method is specific or selective for the pomalidomide.

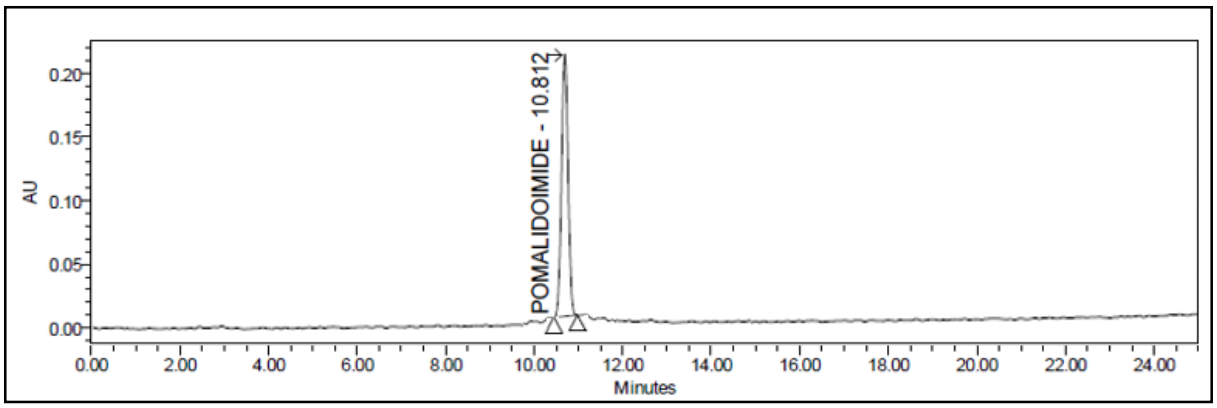

Figure 2: Chromatogram for Standard solution of Pomalidomide

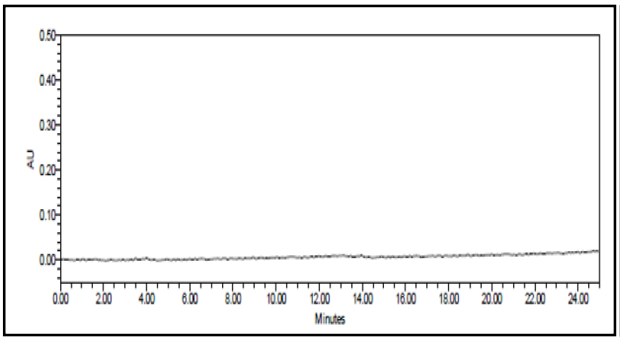

Figure 3: Chromatogram for Blank solution of Pomalidomide

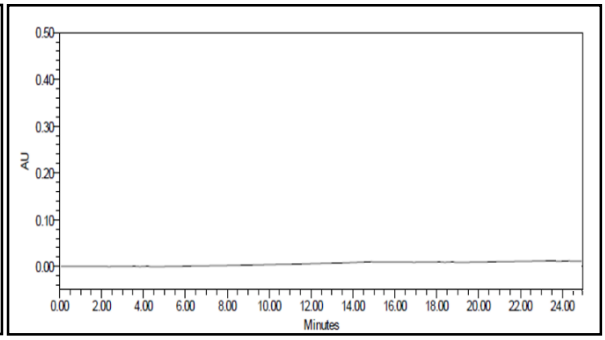

Figure 4: Chromatogram for Placebo solutionof Pomalidomide

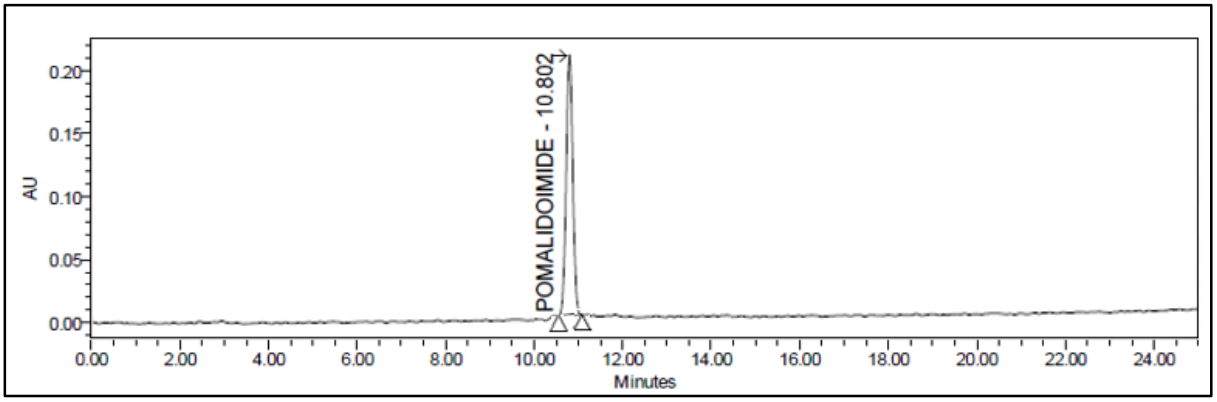

Figure 5: Chromatogram for Sample solution of Pomalidomide

2.3. Linearity:

A linear relationship was evaluated across the range of the analytical procedure, according to label claim solution concentration were prepared. Linearity was evaluated by visual inspection of a plot of signals as a function of analyte concentration. Data from the regression line itself helpful to provide mathematical estimation of the degree of linearity. The correlation coefficient, y-intercept and slope of the regression line was calculated. Thelinearity of Pomalidomide wasperformed using standard solutions in the range of 15 to 45 µg/mL standard. Each of these drug solutions (10 µL) was injected three times into the system, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector. The calibration graph was obtained by plotting peak area versus concentration in µg/mL of Pomalidomide. The plot was found to be linear with correlation coefficient of 0.9984. The respective linear regression equation being Y =77701X+7076.6. Thecorrelationcoefficient('R^2')was notlessthan0.99 hence method is linear and can be consider as a new assay method for quantitative estimation of Pomalidomide. **[10-12]**

Table No. 2- Linearity data table for Pomalidomide

Level	**Concentration (µg/ml)**	**Mean Peak Areas**
50%	15	1156228
75%	22.5	1809339
100%	30	2292452
125%	37.5	2915565
150%	45	1131603
Slope	77701	
Intercept	7076.6	
Regression	0.9984	

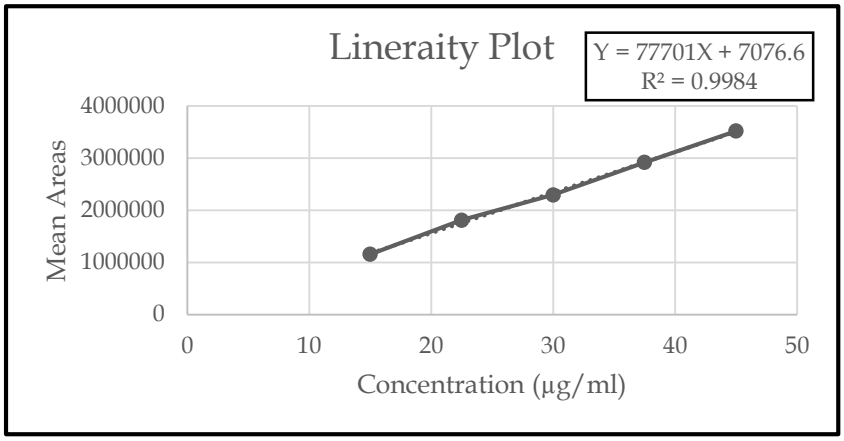

Figure 6- Linearity plot for Pomalidomide

2.4. Accuracy:

Accuracy study is also denoted as recovery studies for the drug using developed analytical method. Pomalidomide sample was spiked with the known amount of Pomalidomide standard solution at 80%, 100% and 120% of test concentration of the drug. The amount of Pomalidomide was quantified as per the developed test method. The percentage recovery was calculated, this test was performed in triplicates. Acceptance criteria for the % Recovery of Pomalidomide at each level and overall average % recovery was in between 98 % to 102% as well as % RSD of Pomalidomide at each level with overall average %RSD was not more than 2.0% gets fulfill hence method is accurate for the quantitative estimation of drug. [10-12]

Table No. 3- Accuracy data table for Pomalidomide

Level (%)	Actual amount added in ppm	Actual amount recovered in ppm	% Recovery	Mean % Recovery	SD#	%RSD*
80	24	23.68	98.67	99.21	0.1353	0.57
	24	23.95	99.79			
	24	23.80	99.17			
100	30	29.68	98.93	98.87	0.1609	0.54
	30	29.81	99.37			
	30	29.49	98.30			
120	36	35.71	99.19	99.54	0.1305	0.36
	36	35.82	99.50			
	36	35.97	99.92			
Overall Mean % Recovery				99.21		
Overall %RSD*				0.49		

* Relative Standard Deviation
# Standard Deviation

2.5. Precision:

In precision method and system was challenged by the preparations of standard solution and sample solution with six replicate injections made of same method of preparation.

a) System precision

Six replicate injections of the standard (30 ppm, using API working standard) were injected into the HPLC system as per the developed method. The Mean, standard deviation and %RSD were calculated and that was not more than 2.0% as per acceptance criteria hence system is appropriate for the assay analysis of Pomalidomide. [10-12]

Table No. 4- System precision data table for Pomalidomide

SR. NO.	Peak areas	%Assay
1	2324390	99.4
2	2318981	99.2
3	2296754	98.2
4	2331891	99.7
5	2299893	98.4

6	2298981	98.3
Mean	2311815	98.9
SD#	15140.72	0.6439
%RSD*	0.65	0.65

* Relative Standard Deviation
# Standard Deviation

b) Method precision

Six replicate injections of the sample (30ppm, using formulation i.e., Capsule) were injected into the HPLC system as per the developed method. The Mean, standard deviation and %RSD were calculated. %RSD of six replicates of Pomalidomide peak area was not more than 2.0% as per method acceptance criteria hence it is proved that method is appropriate or precise towards the assay analysis of Pomalidomide. [10-12]

Table No. 5- Method precision data table for Pomalidomide

SR. NO.	Peak areas	%Assay
1	2298860	98.3
2	2315954	99.0
3	2310284	98.8
4	2320265	99.2
5	2329571	99.6
6	2298781	98.3
Mean	2312286	98.9
SD#	12186.39	0.5125
%RSD*	0.53	0.52

* Relative Standard Deviation
# Standard Deviation

2.6. Robustness:

The robustness of a proposed analytical method was carried out to analyze that the method remained unaffected by small but deliberate or intentional changes in method parameters and provides an indication of its reliability during routine analysis for quantitation of a pomalidomide. The standard solution was injected six times for each varied conditions Change in flow rate (±0.2 ml/min), Change in column temperature (±2°C), Change in wavelength (±2 nm) as mentioned in table no.6, then chromatograms were recorded. The variation had no significant effect on the retention time and chromatographic response of the method, indicating that the assay method is robust. [10-12]

Table No. 6- Robustness Data Table for Pomalidomide

Sr. No.	Flow rate (0.8 mL/min)	Flow rate (1.2 mL/min)	Column oven temperature (23°c)	Column oven temperature (27°c)	Low wavelength (223nm)	Low wavelength (227nm)
1	2305025	2315861	2304232	2283250	2308131	2308865
2	2285323	2289254	2271049	2316281	2280384	2289892
3	2314951	2312334	2282698	2312514	2292952	2319765
Mean	2301766	2305816	2285993	2304015	2293822	2306174
SD#	15080.41	14451.40	16835.10	18081.39	13893.96	15117.21
%RSD*	0.66	0.63	0.74	0.78	0.61	0.66

* Relative Standard Deviation,
# Standard Deviation

3. DISCUSSION

A simple, specific, sensitive RP-HPLC method is developed in this research for the quantification of pomalidomide in pharmaceutical solid dosage form which is Hard shell capsule. The main advantages of this method include it's considerably shorter run time with gradient mobile phase flow which provides sensitivity and specificity of analytical method towards drug with minimum amount of the sample, all the preparations like mobile phase, diluent, sample as well as standard preparations are easy and provides simplicity towards understanding the method with cost efficient manner. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed with accuracy. The absence of extra peaks other than main peaks in the chromatogram indicates non-interference of the common excipients of the capsules. The results of

validation tests were collectively, indicative for a method with a relatively wide linear range, acceptable precision, accuracy, robustness and practically reliable sensitivity.

4. CONCLUSION

The method enables analysis of pomalidomide capsules and can be used for routine analysis in pharmaceutical quality control as well as it can provide base towards further method development for another analysis within a short time. Proposed RP-HPLC strategy promotes amazing affectability, accuracy and reproducibility withminimum sample requirement than the methods which are reported until date for both bulk drug as well as formulation.

5. MATERIALS AND METHODS

5.1. Materials:

Acetonitrile, Potassium Di hydrogen phosphate and ortho-phosphoric acid, Methanol used were of HPLC grade purchased from Sigma-Aldrich (st. Louis,USA), Commercially available Pomalidomide Capsules 2 mg Pomalid® 2 mg, were procured from local market, A millipore Milli-Q-ultrapure water system (Milipore, Australia) was used to obtain highly pure water, Pomalidomide reference standard was obtained as a gift sample from Enaltec Pharma. Research Pvt. Ltd.

5.2. Instrument:

Quantitative HPLC was performed on the Waters Alliance 2695 Separations Module is a High-Performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing with auto-sampler and programmable temperature control. The detector is a photodiode array of model 2996. The HPLC system was equipped with Empower-3 solution software.

5.3. HPLC Conditions:

Column- Kinetex Phenyl Hexyl XB-C18, (250x4.6mm, 5µm particle size)
Mobile phase- Buffer and Methanol.
Mobile phase-A: Buffer (pH to 3.0±0.05.)
Mobile phase-B: Methanol (100%) in gradient mode of elution shown in Table.
Gradient program –

Table No. 7: Gradient Program

Time (Min)	Solvent-A (%)	Solvent B (%)
0.0	65	35
20	40	60
21	50	50
25	65	35

Detection Wavelength: 225nm., Flow rate – 1.0 ml/min., Run time – 25 min., Temperature- Ambient, Injection volume-10µl., Retention time -09 to 13 min.

Diluent-
Diluent-1- 0.1% Ortho Phosphoric Acid (Add 1 ml of Orthophosphoric acid 85% in 1000 ml water and mix well).
Diluent-2- 0.1 % OPA: Acetonitrile in the ratio of 80:20

5.4. Preparations:

Preparation of Standard stock solution:

A standard stock solution of the drug was prepared by dissolving 30 mg of Pomalidomide in 100 ml volumetric flask containing 50 ml of diluent-2 with gradual addition, sonicated for about 15 minutes and then made up to 100 ml with diluent-2 to get approximately 300µg/ml.
(Concentration of Pomalidomide is 300 ppm).

Preparation of Working Standard Solution:

5ml of the primary standard stock solution of 300µg/ml was taken in 50 ml volumetric flask and thereafter made up to 50 ml with diluent-2 to get a concentration of 30µg/ml.

Preparation of Sample solution:

Each 10 capsules of Pomalid* 2 mg were weighed and content were emptied and transferred into dry watch glass. Capsule powder equivalent to 30 mg of pomalidomide and transfer into 100 ml volumetric flask, add about 40 ml of diluent-1 sonicate for 10 minutes with intermediate shaking then add about 40 ml of Acetonitrile sonicate for 10 minutes with intermediate shaking, cool the flask and dilute upto the mark with same Acetonitrile and mix well. Filter the solution through 0.45µ Nylon filter with discarding initial 3 ml filtrate. Further dilute 5.0 ml of this solution to 50 ml with the diluent and mix. (Concentration of Pomalidomide is 30 ppm).

Preparation of Mobile phase:

Mobile phase used is Buffer: Methanol.
Mobile phase-A: Buffer (1.36 gm of Potassium Di hydrogen ortho-phosphate (0.02M) in 1000 ml of

water and by adjusting the pH to 3.0±0.05 with dilute orthophosphoric acid.)
Mobile phase-B: Methanol (100%) in gradient mode of elution was used to resolute the Pomalidomide. Mobile phases were filtered through a 0.45 µm membrane filter before use and degassed using sonication.

Conflict of interest:
The authors have no conflicts of interest regarding this investigation**.**

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