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A Study on Micellar Liquid Chromatographic Analysis of Multivariate Forced Degradation of Chemotherapeutic Drug Irinotecan Using an Experimental Design Approach: Response Surface Methodology

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Abstract:	Irinotecan is a chemotherapeutic agent and one of the most commonly prescribed drugs internationally. Every drug that is commercialized should pass through some quality control parameters. To meet this requirement some screening techniques are used and chromatography is one of them. In the present research work, a chromatographic method has been developed where surfactant, short-chain alcohol and water is used as a mobile phase, known as micellar liquid chromatographic method coupled with a photodiode array detector (MLC-PDA). The developed method is easy to perform, rapid, cost-effective and sensitive. The efficiency and fastness of the method can be improved by adding Response Surface Methodology (RSM) with a central composite design (CCD) approach because it reduces the number of experiments being performed on the basis of experimental design. The method can be easily applied to detect irinotecan in different degradation conditions (domestic light source, acid and alkali, oxidative). Here, a mobile phase of 0.15 M SDS 6% pentanol pH 7 was eluted under isocratic mode from C18 column in which irinotecan was eluted within 6 min. The method has been validated following the ICH guidelines. The influence of SDS, organic modifier, 1-pentanol (OM) and pH on retention time (Rt) was established using RSM-CCD. The correlation coefficient (r2) value was 0.999 in the linear range of 0.25–30µg/mL. The detection and quantification limits for irinotecan are 0.20 and 0.25µg/mL, respectively. Repeatability and intermediate precision below 6.03% were adequate for the quantification of irinotecan in degradation conditions. The developed method was successfully applied to estimate irinotecan in different degradation conditions.
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Editor

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Dear Sir,

We enclose hereby the manuscript entitled,“ **A Study on Micellar Liquid Chromatographic Analysis of Multivariate Forced Degradation of Chemotherapeutic Drug Irinotecan Using an Experimental Design Approach: Response Surface Methodology”** to be considered for publication in **Journal of Pharmaceutical and Biomedical Analysis** in special issue on ***Selected Papers from Recent Developments in Pharmaceutical Analysis 2023 (RDPA2023)”.***

Irinotecan is a widely prescribed medication to manage and treat a variety of solid tumours. It is in the DNA topoisomerase I inhibitor class of drugs and used adjunctively with other therapeutic agents against colorectal cancer as a first- or second-line treatment. Multivariate degradation and analytical method validation are important for quality control procedures in the pharmaceutical industry and for ensuring the clinical success of the therapy. For method validation, as many of the analytical methods are based on RP-HPLC, which uses large quantities of hazardous organic solvents, we require an alternative less contaminant but maintains its advantages. Besides, the study of the theoretical background of retention is also interesting. Apart from that the degradation study using a domestic light source and acid, alkali with validation also emphasized the work in one article. The paper describes developing a method based on micellar liquid chromatography to quantify irinotecan in a multivariate degradation study under different conditions. The main feature of validation is using response surface methodology (RSM) with central composite design (CCD). The second-order polynomial model for predicting the optimal chromatographic run time was evaluated by the analysis of variance (ANOVA) and 3D response surface plots for the interactions between three variables were constructed. Here only one solution for the entire analysis (sample processing and chromatographic analysis), which contains only a low proportion of 1-pentanol (less hazardous than methanol or acetonitrile). Besides, the analyte is resolved in <6.0 min and the

procedure exhibits a high sample throughput. The procedure uses basic laboratory resources and low manpower. The method was carefully and successfully validated according to official guidelines. The procedure exhibited enough sensitivity for the intended purpose of the analysis. The method was demonstrated to be short-term, easy to handle, practical, reliable, safe, green, and cost-effective compared to hydro-organic RP-HPLC. These performances of the method make it an excellent choice for pharmaceutical routine analysis.

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We look forward to your answer.

With regards,

Abhilasha Durgbanshi

Highlights

- A green MLC-PDA method optimized with the aid of RSM.
- The developed method was applied for the degradation of the chemotherapeutic drug irinotecan.
- The number of pretreatment steps was reduced in comparison to LC-MS and HPLC, and the volume of organic solvent used during analysis was also reduced.
- Chromatographic run time was < 6 min.
- Validated following ICH international guidelines.
- Percentages of degradation were reported under different conditions

A Study on Micellar Liquid Chromatographic Analysis of Multivariate Forced Degradation of Chemotherapeutic Drug Irinotecan Using an Experimental Design Approach: Response Surface Methodology

Abstract:

Irinotecan is a chemotherapeutic agent and one of the most commonly prescribed drugs internationally. Every drug that is commercialized should pass through some quality control parameters. To meet this requirement some screening techniques are used and chromatography is one of them. In the present research work, a chromatographic method has been developed where surfactant, short-chain alcohol and water is used as a mobile phase, known as micellar liquid chromatographic method coupled with a photodiode array detector (MLC-PDA). The developed method is easy to perform, rapid, cost-effective and sensitive. The efficiency and fastness of the method can be improved by adding Response Surface Methodology (RSM) with a central composite design (CCD) approach because it reduces the number of experiments being performed on the basis of experimental design. The method can be easily applied to detect irinotecan in different degradation conditions (domestic light source, acid and alkali, oxidative). Here, a mobile phase of 0.15 M SDS 6 % pentanol pH 7 was eluted under isocratic mode from C_{18} column in which irinotecan was eluted within 6 min. The method has been validated following the ICH guidelines. The influence of SDS, organic modifier, 1-pentanol (OM) and pH on retention time (Rt) was established using RSM-CCD. The correlation coefficient (r^2) value was 0.999 in the linear range of 0.25–30 µg/mL. The detection and quantification limits for irinotecan are 0.20 and 0.25 µg/mL, respectively. Repeatability and intermediate precision below 6.03% were adequate for the quantification of irinotecan in degradation conditions. The developed method was successfully applied to estimate irinotecan in different degradation conditions.

Keywords: I*rinotecan, micellar liquid chromatography, response surface methodology, central composite design, validation, degradation*

1. Introduction

Irinotecan is a chemotherapeutic drug which either alone or in combination with other antitumour drugs is used to treat lung cancer or colorectal cancer. It is derived from camptothecin (alkaloid extracted from a tree namely Campthotheca Acuminata) that constrains topoisomerase action and prevents relegation of DNA strands [1]. This drug was approved in the year of 2015 by the Food and Drug Administration for the treatment of advanced pancreatic cancer under the trade name Onivyde [2]. Since then, it has been continuously used as a chemotherapy agent against different tumours like colorectal, pancreatic, ovarian and lung [3].

The degradation study of the drugs is an essential aspect of knowing the stability of the pharmaceutical molecule. Significant stability-testing analytical techniques are generally needed in the modern analytical laboratory. Environmental factors that pose significant effects on the stability of pharmaceutical substances include light, temperature, pH, oxygen, moistness, additives, excipients, etc [4,5]. Stress testing is a useful tool for determining degradation products and can reveal crucial details on the inherent stability of pharmaceutical compounds [6,7]. As per the ICH drug degradation study, the main aim of drug stability testing is to know its effective shelf life and suggest where it can be stored [8,9].

Due to the structural characteristics of irinotecan, it can transform into CPT-11 [10], N-38 and PDP (photodegradation products) [11]. Available literature of irinotecan in which the degradation study being studied by liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC) shows that different potential degradation products are formed with closely related process impurities that could be found in the end product of drugs due to the manufacturing process [12,13].

In chromatography, mobile phase optimization is a very important step for detecting and quantifying analyte of interest in a sample matrix with suitable separation parameters. However, the traditional method of mobile phase optimization takes a long period and a lot of

experimentation to determine ideal levels. In order to, optimize the chromatographic method with minimum time and with good precision, researchers are applying some mathematical models and response surface methodology (RSM) is one of them. This model employed an iterative process involving trials with the best mobile phase, fitting an experimental RSM model, and refining the estimated response model. This is because when a method is optimized using a traditional process in which one factor is varied and other factors are kept at fixed levels, it may not represent the aggregate effect of all components working for peak parameters. RSM offers several advantages over the traditional optimization method, including statistically analyzed data, interactions between experimental parameters, the need for minimum experiments, and a reduced operating time. These designs need three levels for each component, and they are rotating or almost rotational.

Researchers have conducted a degradation study of irinotecan using LC-MS [14] and HPLC [15], but both of these chromatographic techniques have their own advantages and disadvantages. LC-MS is a costly technique that needs sophisticated maintenance. On the other hand, HPLC uses toxic solvents, which are not good for the environment or the operator. In the present research work, RSM, commonly known as the experimental design approach, has been utilized to optimize and validate micellar liquid chromatography coupled to photodiode array detector (MLC-PDA) for the detection of degradation product of irinotecan in different conditions. MLC is a mode of reversed-phase liquid chromatography in which surfactant is used as a mobile phase component above its critical micellar concentration [16,17].

It uses a significantly less amount of organic modifiers, mainly short-chain alcohols. The developed method follows the principles of green chemistry, making the study more eco-friendly than other chromatographic methods [18]. After optimization, the MLC method was applied to determine the quantity and rate of formation of other components upon photocatalytic degradation of irinotecan within stipulated time intervals in acid (HCl), alkali

(NaOH) common domestic light sources (dark, tungsten and white and blue LED light) and with 3% H_2O_2 solution [19]. The selected parameters or conditions were similar to those used by A.Rajasekaran et al., with modifications [15]. As the MLC technique has several advantages over other chromatographic techniques [20], it could easily be applied to forced degraded samples of irinotecan.

2. Experimental section

2.1. Chemical and reagents

The irinotecan standard was gifted by OREX PHARMA Pvt. Ltd. (Mumbai, Maharashtra). Sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) and sodium dodecyl sulphate (SDS;99%) were procured from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Hydrochloric acid (HCl ≈ 37%), sodium hydroxide (NaOH >99.0%), hydrogen peroxide (H_2O_2 >98.9%), 1-propanol, 1-butanol, and 1-pentanol were of HPLC grade and purchased from Rankem, RFCL Limited (New Delhi, India).

2.2. Chromatographic system and conditions

The used chromatographic system was Shimadzu Prominence, Kyoto (Japan), equipped with an isocratic pump (LC-20 AT), autosampler (SIL-20AC) and a photodiode array detector (SPD M20A190-800nm). The column was a Shimadzu C_{18} analytical column (250 mm length × 4.6 mm with 5 µm particle size) and the injection volume was 20 µL with flow rate 1 mL/min. All the chromatographic data were acquired and processed with the help of Shimadzu LC Solution software version 1.22 SP1.

Mettler-Toledo analytical balance (ME204) delivered by Pocklington, United Kingdom. The magnetic stirrer and ultrasonic bath were of PCI Analytics. pH meter (Contech LAB, Model pH-103) and ultrapure Type-I water (LAB Q Ultra) were purchased from

Mumbai, India. Membrane filters of 0.45 µm (Micron Separations) size were procured from Westboro, USA. The maximum absorption wavelength of irinotecan was 268 nm.

2.3. Mobile phase and standard solution preparation

The micellar mobile phases were prepared using different combinations of SDS, organic modifier, and ultrapure Type-I water. Then, they were buffered using 0.01 M $NaH_2PO_4.H_2O$ at pH 7 using either HCl or NaOH (0.1 M) solutions with a continued stirrer. After that, a required volume of 1-propanol, 1-butanol, and 1-pentanol (organic modifiers) was added one at a time, and the volumetric flask was filled up to the mark using ultrapure Type-I water. Lastly, the mobile phase was sonicated for 10 min. to solubilize all the components and filtered using a 0.45 µm nylon membrane filter, with the aid of a vacuum pump to remove solid particles.

The irinotecan stock solution (100 ppm) was prepared by solubilizing it in the methanol-water mixture (1:9) with the help of an ultrasonic bath and stored in a refrigerated condition at 4 °C.

2.4. Forced degradation study

Irinotecan was exposed to conditions of photolysis, acid degradation and alkali degradation. The concentration of irinotecan in solution for the study was 20 µg/mL. A conical flask containing 25 mL irinotecan samples was kept in the dark, tungsten light, LED white and LED blue light chamber 30 cm away from the direct light source. At the time intervals of 3, 6 and 24 hours, 2 mL samples were withdrawn and immediately injected into the chromatographic system after filtration. The temperature changes were also documented during this experiment, which is presented in **Table 1**. Solutions of irinotecan in NaOH (0.1 M) and HCl (0.1 M) were exposed to 80 °C for 4 hours for acidic and alkaline degradation and after that both solutions were cooled. In case of acidic degradation, the obtained solution was

neutralized with 5 mL of HCl (0.1M), while in case of basic degradation, 5 mL of NaOH (0.1M) was added and both the samples were made 25 mL by adding 14 mL of ultrapure Type-I water. For the oxidative stress study, 1 mL of standard irinotecan solution was mixed with 9 mL of 3% H_2O_2 (hydrogen peroxide) solution.

3. Result and discussion

3.1. Mobile phase variable selection

The present experiment work focused on optimizing simple, rapid and cost-effective mobile phases for satisfactory results. The mobile phase utilized in MLC is composed of bulk water, a smaller amount of SDS, Na_2HPO_4 and organic modifiers. Surfactants used in the MLC system are Brij-35, SDS and CTAB. Among all these surfactants, SDS has been used based on existing literature [24]. Generally, short-chain alcohols i.e.1-propanol, 1-butanol, 1-pentanol, are added in the mobile phase for the purpose of improved efficiency and suitable elution time of the analyte. The Po/w value of irinotecan is 3.0, which shows it is hydrophobic and the use of 1-propanol will help to improve the peak parameters like asymmetry factor, efficiency, capacity factor etc. As the analyte is hydrophobic in nature, it will elute late in pure SDS, consequently causing long retention time (Rt) and poor peak parameters. Replacing 1-propanol with other higher alcohols, i.e. 1-butanol or 1-pentanol, will result in a suitable Rt and the acceptance of peak parameters for irinotecan. Therefore, in this work, 1-pentanol was utilized as an organic modifier. To optimize the concentration of SDS and the volume of 1-pentanol response, surface methodology (RSM) was applied with adapted pH. A total of 20 mobile phases were prepared with different concentrations of SDS in the range of 0.05-0.15 M and 1-pentanol i.e. organic modifier in the range of 2-6 % and pH from 3-7.

3.2. Experimental studies and data analysis

3.2.1. Response surface methodology

Early chromatographic technique optimization procedures involved fixing all of the independent variables before modifying one at a time to see the effect on the run time of chromatography and other parameters. This mobile phase optimization approach does not present the interactive effect of all independent variables since the modification only influences one independent variable. The traditional method of mobile phase optimization is lengthy and laborious and requires more solvents because it requires more experiments. This can be minimized using experimental designs such as RSM.

The effect of selected variables was determined on the Rt of irinotecan using RSM, and the separation variables investigated were SDS, 1-pentanol, and pH of the mobile phase. As presented in **Table 2,** experimental studies were started by creating a central composite design (CCD), which involved a total of 20 runs (Table 3), with 13 axial points and 7 central points with a rotatable design. Selected variables were calculated at five levels (−1.68, −1, 0, +1, +1.68) by fixing the alpha value at 1.68. The CCD result was the retention time of irinotecan. In the quadratic polynomial model, experimental values were placed to achieve regression coefficient value. The model (quadratic polynomial) applied in the response surface analysis is:

$$x = a_0+a_1b_1+a_2b_2+a_3b_3+a_{11}b_{12}+a_{22}b_{22}+a_{33}b_{32}+ a_{12}b_1b_2+a_{13}b_1b_3+a_{23}b_2b_3+a_{123}b_1b_2 \quad \textbf{Eq. (1)}$$

Where “x” represents retention time, a_0 is a constant for the line intersecting the b axis, a_1, a_2, a_3 a_{12}, a_{13}, a_{23} and a_{123} are coefficients of regression [21,22]. The relationship between the retention time and levels of each variable was revealed by a polynomial equation in the form of a three-dimensional surface plot using design expert software.

3.2.1. validation of proposed model and statistical evaluation

The optimization tactic generally starts with setting up a regression model between pH,

SDS and 1-pentanol of the mobile phase (independent) and Rt of irinotecan(dependent variables). In the present research work, a quadratic polynomial model with regression coefficient and F-value were used to achieve the minimum retention time of the analyte under the optimized chromatographic condition. Therefore, ANOVA (variance analysis) and other statistical parameters of the model, as shown in Table 4, were analyzed for retention time. Statistical variables used for the polynomial model (quadratic) have all variables (pH, SDS, OM), their interactive variables (SDS ×pH, SDS ×OM, OM × pH), and self-interactive variables (pH^2, SDS^2, OM^2,). Here the following equation was used to represent all these variables:

$$Rt = +20.374+ 78.7\ SDS -1.641\ pH - 1.359\ OM + 0.625\ SDS \times pH + 1.125\ SDS \times OM + 0.0281\ pH \times OM + 146.18SDS^2 + 0.095\ pH^2 +0.078\ OM^2 \quad \textbf{Eq. (2)}$$

The obtained values of P and F from the ANOVA examination showed that the full model was valid and properly explained the key response (i.e., analyte retention time).

In **Figure 1**, the high effect of SDS concentration, mobile pH, and % of organic modifier (1-pentanol) on the Rt of irinotecan has been shown. Based on the graph, these parameters were the best suited to achieve the lowest Rt with increased efficiency. The obtained F-value of ANOVA analysis for the selected model is 215.5 which shows that the method fits well. In addition, the CCD model is substantial and could be applied for all response values under experimental settings. The regression coefficient is one of the critical statistical variables of the model which can be employed for model validation (R^2). Here, **Table 4** contains various regression statistical values, including the regression coefficient of prediction (R^2 pred.) and the adjusted R^2 (R^2 adj.), which demonstrate the suggested fitness of the model and predictive power, respectively.

3.2.3. Graph evaluation

The selected separation variable effect on Rt is graphically presented in **Figure 2**. In this study, graphs were plotted Rt and two independent variables by keeping one-factor constant.

Effect of SDS and OM

Here, the response surface graph (**Figure 2a**) shows that increasing the concentration of SDS has an important effect and it affects irinotecan Rt. The minimum Rt was achieved when a high concentration of SDS (0.15 M) was used because the slope of the curve was steeper toward SDS axis (**Figure 2a**). The graph also showed that OM remarkably affects the response value.

Effect of pH and SDS

During the preliminary examination, it was observed that pH had a major effect on the retention time of irinotecan because it was neutral in the selected pH range (based on pKa value) but due to the cationic nature of noticeable variation in retention time was observed for it, which varies as; 8.9 (pH 3), 10 (pH 5) and 5.1 (pH 7). As depicted in **Figure 2b,** minimum retention time was obtained using the highest pH value (in the range of 2-7) and SDS concentration.

Effect of pH and OM

The effect of 1-pentanol was studied in preliminary experimental analysis to select a suitable concentration of it. **Figure 2c** presents the combined effect of pH and OM concentration on the retention time of irinotecan. Here, it has been shown that using a higher pH value and higher concentration of OM (6%), a minimum Rt was obtained.

3.3. Method validation

The ICH guidelines have been used and validated for irinotecan. Different validation characteristics have been addressed for this study, such as selectivity, linearity, accuracy, precision, limit of detection and quantification and robustness [23,24].

3.3.1. Selectivity

The specificity or selectivity of the developed method was examined by obtaining the chromatogram of the standard sample solution of irinotecan and blank chromatogram (only diluent). Specificity signifies the analyte identification in the presence of other interference (matrix). For this purpose, a solvent in which an irinotecan sample was prepared was used as a blank and spiked with 20 µg/mL concentration of standard irinotecan solution (**Figure 3**). The blank chromatogram shows the interference peak up to 2.5 min., after that, there were no peaks.

3.3.2. Linearity

Linearity of the sample solutions were prepared using stock solutions of irinotecan with ultrapure Type-I water to obtain different concentrations. The dilutions were prepared from 0.25-30 µg/mL and plotted between the obtained area and analyte concentrations. The samples were injected three times for each concentration under the same environments. The linear regression model has been used to calculate the linearity of the calibration curve by applying least square linear regression method. The slope, correlation coefficient, y-intercept, and relative standard deviation of the calibration curve are presented in **Table 5**.

3.3.3. precision and accuracy

The system's precision (intraday) and accuracy (interday) were determined by analyzing irinotecan at 3 concentrations (1, 2.5 and 5 µg/mL). In the case of intraday precision, the investigation was performed by injecting three concentrations of sample solutions five times on the same day. While in the case of interday analysis, it was the average of five measurements of the intraday values taken on four days over the time period of 4 months and performed by different analysts using same concentrations. For precision, the results were articulated as % of relative standard deviation (RSD) while for accuracy, it was presented as % relative error (**Table 6)**. The obtained values for accuracy (between ε_R2.5 and 11%) and

precision (RSD > 6.03 %) were well below the accepted value as per the guideline (less than 15% for accuracy and precision).

3.3.4. Robustness

The robustness of the validated and developed chromatographic method was determined by incorporating changes in chromatographic conditions i.e. mobile phase which includes SDS concentration (0.14 - 0.16 M), volume % of pentanol (6.9-7.1%) pH (6.9-7.1) and flow rate were slightly changed. With these changes in system suitability parameters for standard irinotecan, the change in chromatographic parameters was recorded. The robustness of the method was less than 7.4 %, which shows that it is robust enough to work out on real samples.

3.4. Degradation study of drug

3.4.1. Photolytic degradation:

The marketed formulation of irinotecan was placed in two conditions i.e. in normal light (placed on a benchtop) and the other was placed in the dark for 24 hours. The solutions stored in dark conditions showed a degradation of 18.7% after 24 hours. However, the colour of the formulation was changed slightly, and it was placed in an open environment, showing around 30.3% degradation (**Figure 4)**. A decrease in measured irinotecan concentration has been reported in the PDA chromatograms. **Figure 5** depicts the degradation of irinotecan in the case of LED blue (5W) or UV light source, and a slight decrease in drug concentration was reported until 24 hours later.

The chromatograms of irinotecan solution exposed to a tungsten filament light of 100 W with time intervals of 3, 6 and 24 hours are presented in **Figure 6**. Irinotecan was not significantly degraded when it was exposed to a tungsten filament light for 3 hours. After 6 hours, the drug was degraded at approximately 19.2% and at 24 hours the degradation of the drug was around 65.9% which shows that the drug was thermo labile because the temperature

of the chamber was also increased to 60°C from 49°C. When the drug was irradiated for 24 hour the degradation was around 83.5% and the temperature was also increased to 80°C. The finding shows that the drug is thermal and photolabile.

3.4.2. Acidic and alkaline conditions

During the experimental work it was found that irinotecan is acid labile. The quantity of irinotecan remains after refluxing for 4 hours in 0.1 N HCl was just 0.21% because no parent peak is visible in the chromatogram presented in **Figure 7.**

During analysis, it was found that irinotecan is more stable in alkaline conditions than in acidic conditions. After refluxing the irinotecan solution in 0.1 N NaOH for 4 hours, around 50.6% degradation was observed (**Figure 8**).

3.4.3. Oxidative condition: H_2O_2 induced

Under oxidative stress conditions induced by H_2O_2 at room temperature, irinotecan showed around 46.7 % degradation, indicating that under oxidative stress conditions, irinotecan is also degraded (**Figure 9**).

This suggests that the irinotecan is degradable by oxidation, heat, photolysis, and hydrolysis (acid, base, H_2O_2 and water). The method appears to have allowed for the precise analysis of irinotecan in the presence of its degradation products, as the degradant peaks in all of the aforementioned examples did not interfere with the irinotecan peak.

4. Conclusion

The current work outlines the use of RSM and CCD to optimize the MLC parameters. In order to identify irinotecan in the shortest chromatographic run time, the optimized chromatographic parameters were applied. The proposed design provides an idea of the interactions between selected variables, i.e., SDS, OM, pH, and Rt. The optimal concentrations of the chosen variables are 0.15 M for SDS, 6% for organic modifier (1-pentanol), and pH 7

which detected irinotecan in the chromatographic run time of <6 min. In addition, a comparison was made between the predicted and experimental values for every variable. The observed ones within the experimental values fit the empirical model quite well. Regression analysis with acceptable R^2 values (0.990) and ANOVA F-test (215.50) values s also showed adequate correlation between the selected variables. Thus, the selected model provides an effective, mechanized, and robust method for determining irinotecan in standard compounds and degradation conditions.

Forced degradation studies provide insights about potential degradation pathways/ products of the active substances, which aid in the determination of the degradants. In the present research work, a forced degradation study was performed to determine the quantity and rate of formation of other components upon photocatalytic degradation of irinotecan within stipulated time intervals in acid (HCl), alkali (NaOH) and common domestic light sources (dark, tungsten and white and blue LED light) and oxidizing agent (H_2O_2). In this work, an MLC method has been developed and validated to test the stability of irinotecan. The established MLC method proved easy, robust, and selective, showing satisfactory accuracy (intraday) and precision (interday). Therefore, it is suitable for routine qualitative as well as quantitative analysis of pharmaceutical compounds. A stability-indicating assay was formulated by following the guidelines outlined by ICH. The sample applied for force degradation shows the formation of other components. This method was designed so that it could easily be opted by pharmaceutical industries, quality control laboratories, and research institutes. It can also be executed for forced degradation study of the selected analyte in different environmental conditions.

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Figure Captions

Figure 1. Scatter plot showing the effect of (a) pH, (b) OM and (c) SDS on Rt of irinotecan.

Figure 2. Three-dimensional (3D) response surface plots showing the effects of (a) SDS vs OM, (b) pH vs SDS, (c) pH vs OM on MLC-PDA method parameters for Rt of irinotecan.

Figure 3. Blank (a) and spiked (b) chromatogram of irinotecan in optimized chromatographic condition.

Figure 4. Dark light (a) and white LED light (b) 24 hours exposed chromatogram of irinotecan in optimized chromatographic condition.

Figure 5. UV 6 hours (a) and UV 24 hours (b) exposed chromatogram of irinotecan in optimized chromatographic condition.

Figure 6. Tungsten 3 hours (a) 6 hours (b) and 24 hours (c) exposed chromatogram of irinotecan in optimized chromatographic condition.

Figure 7. Acidic 4 hours exposed chromatogram of irinotecan in optimized chromatographic condition.

Figure 8. Alkaline 4 hours exposed chromatogram of irinotecan in optimized chromatographic condition.

Figure 9. Hydrogen peroxide 24 hours exposed chromatogram of irinotecan in optimized chromatographic condition.

Figure 1a

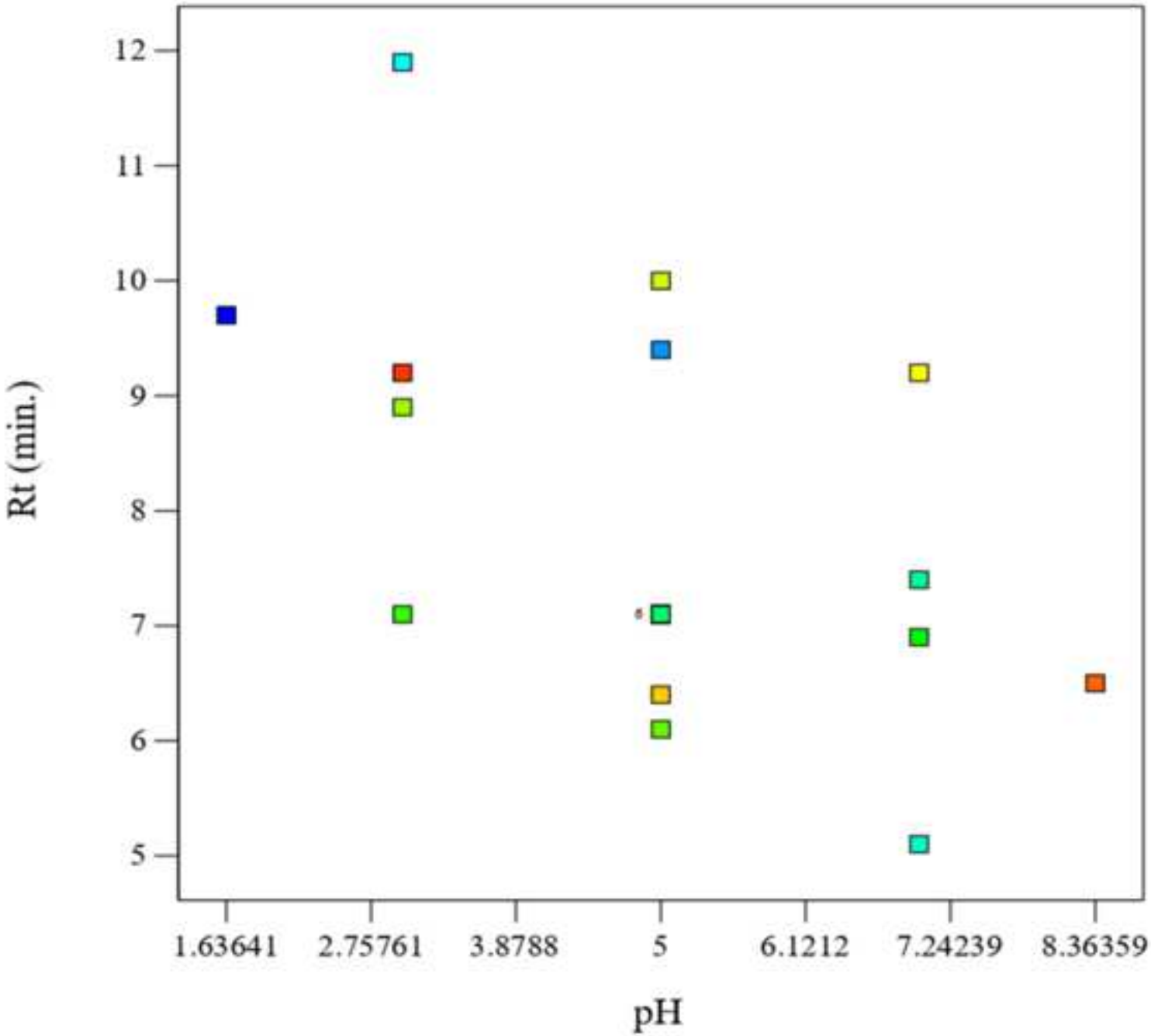

Figure 1b

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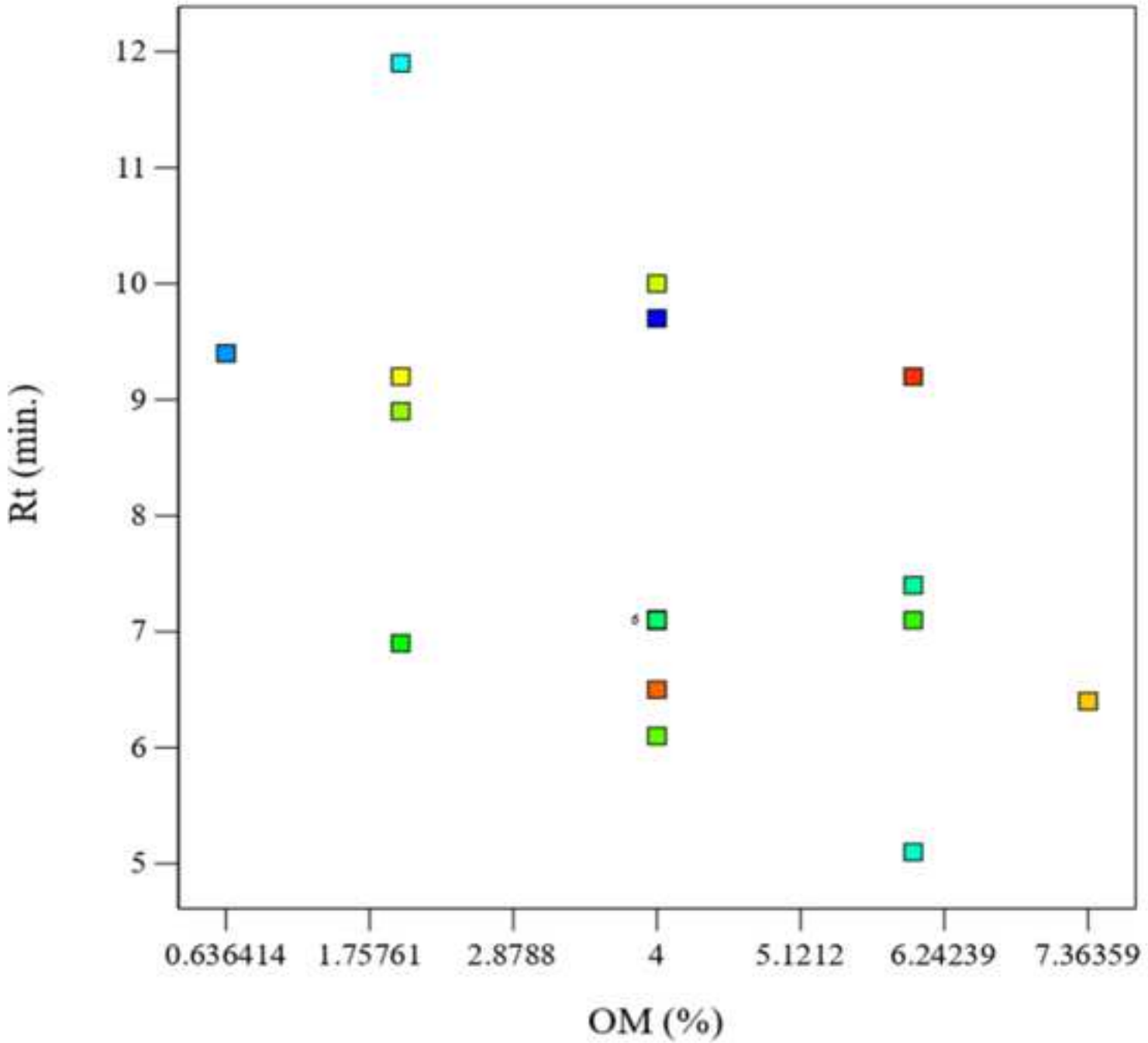

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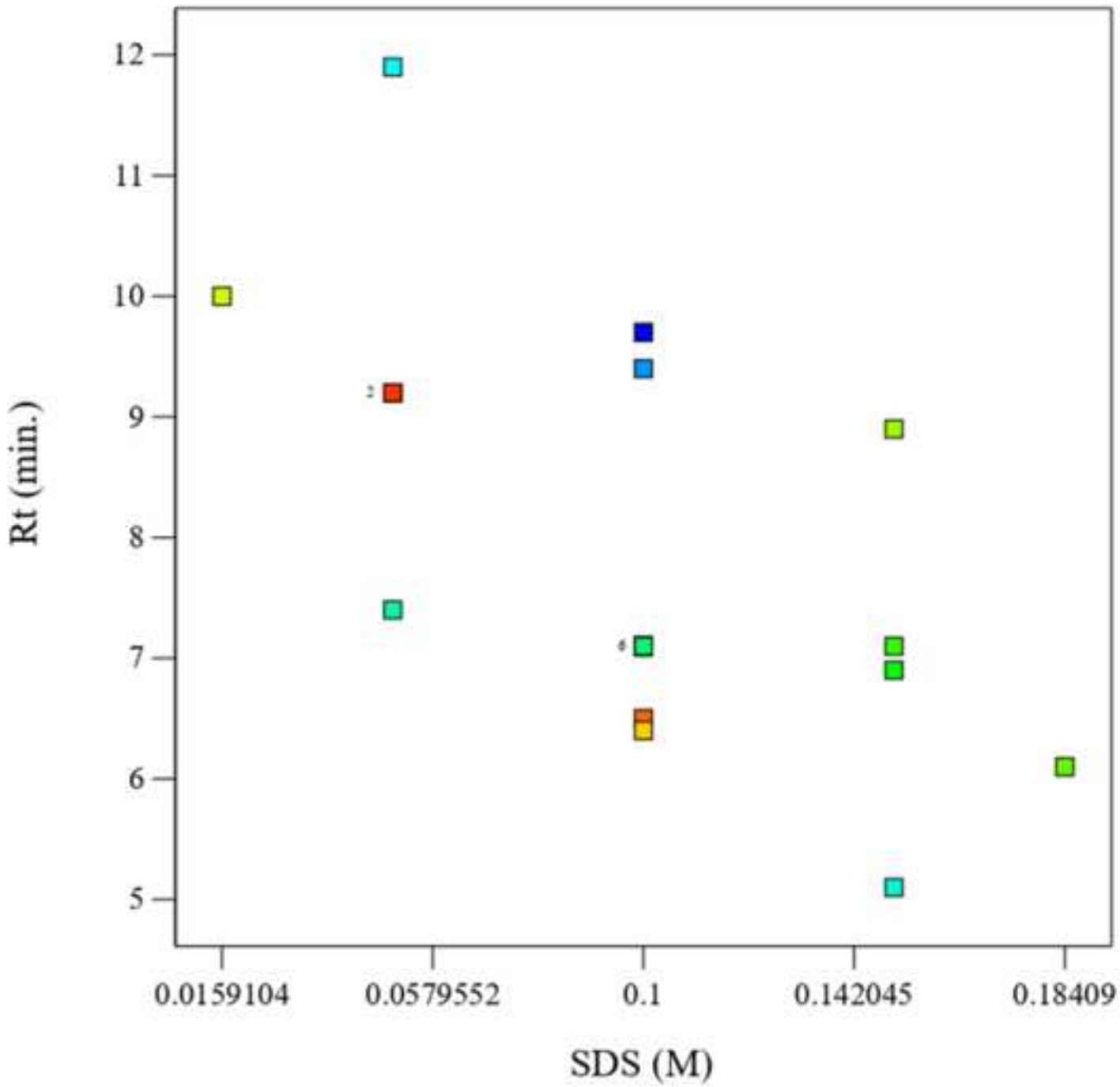

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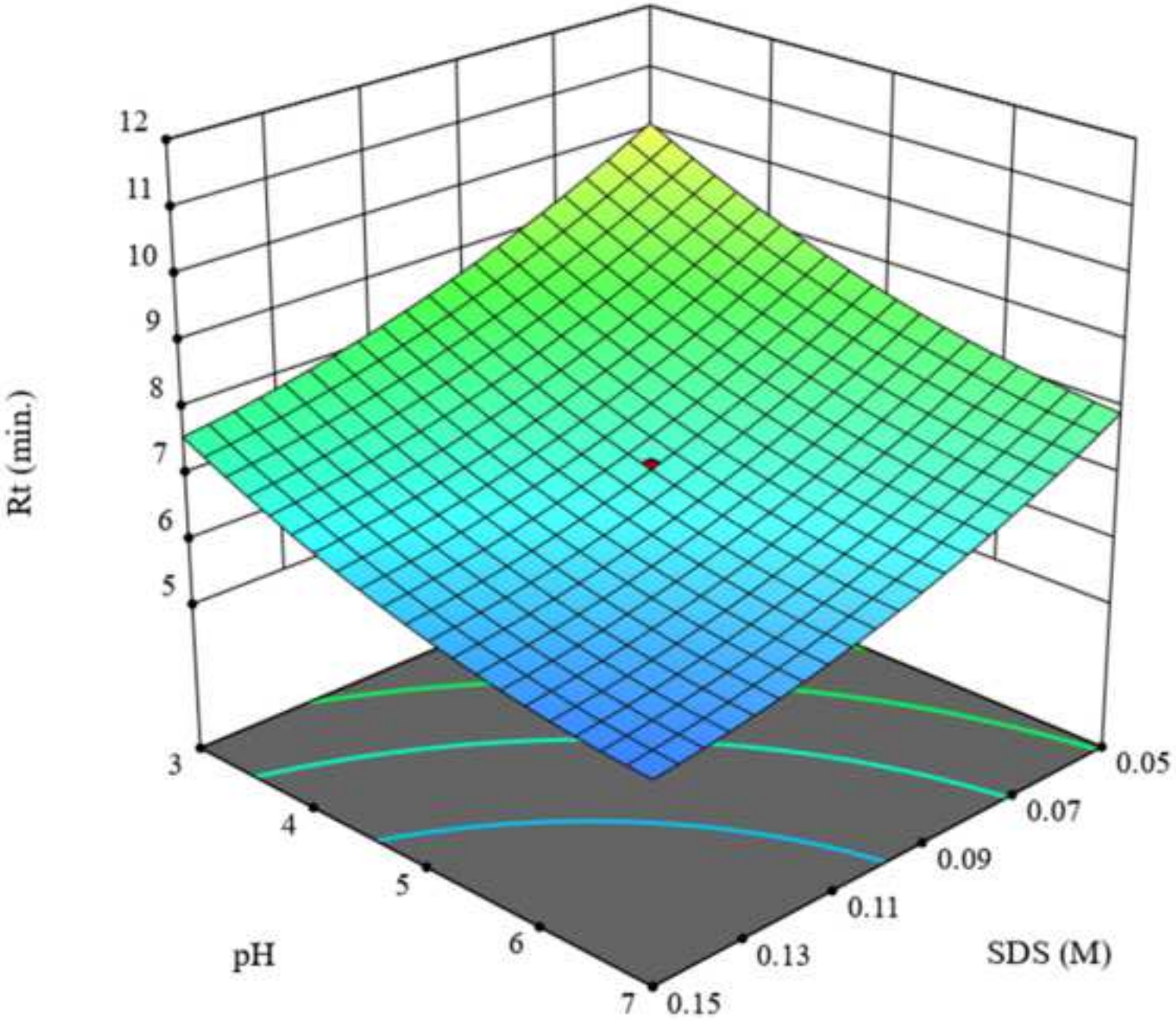

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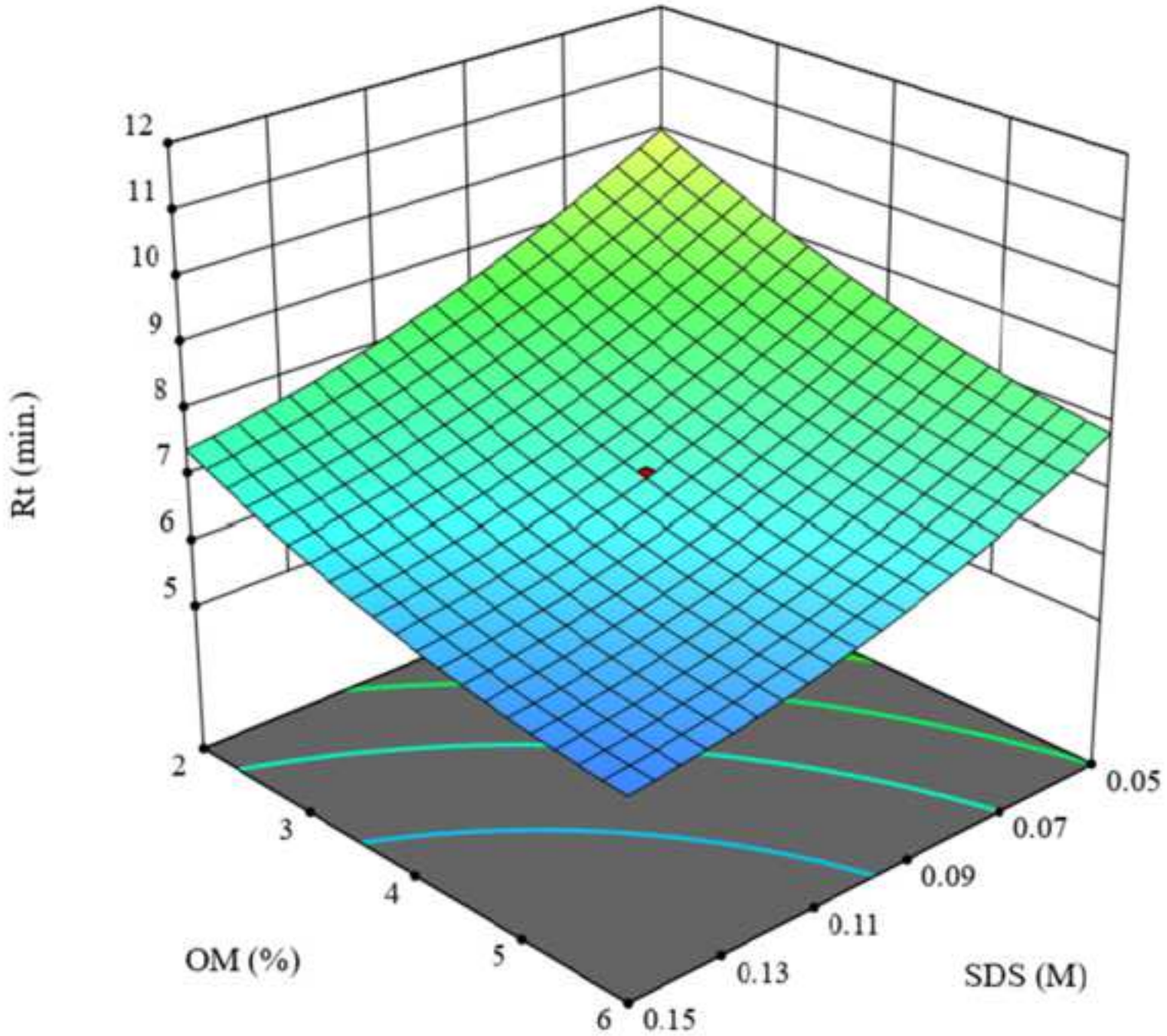

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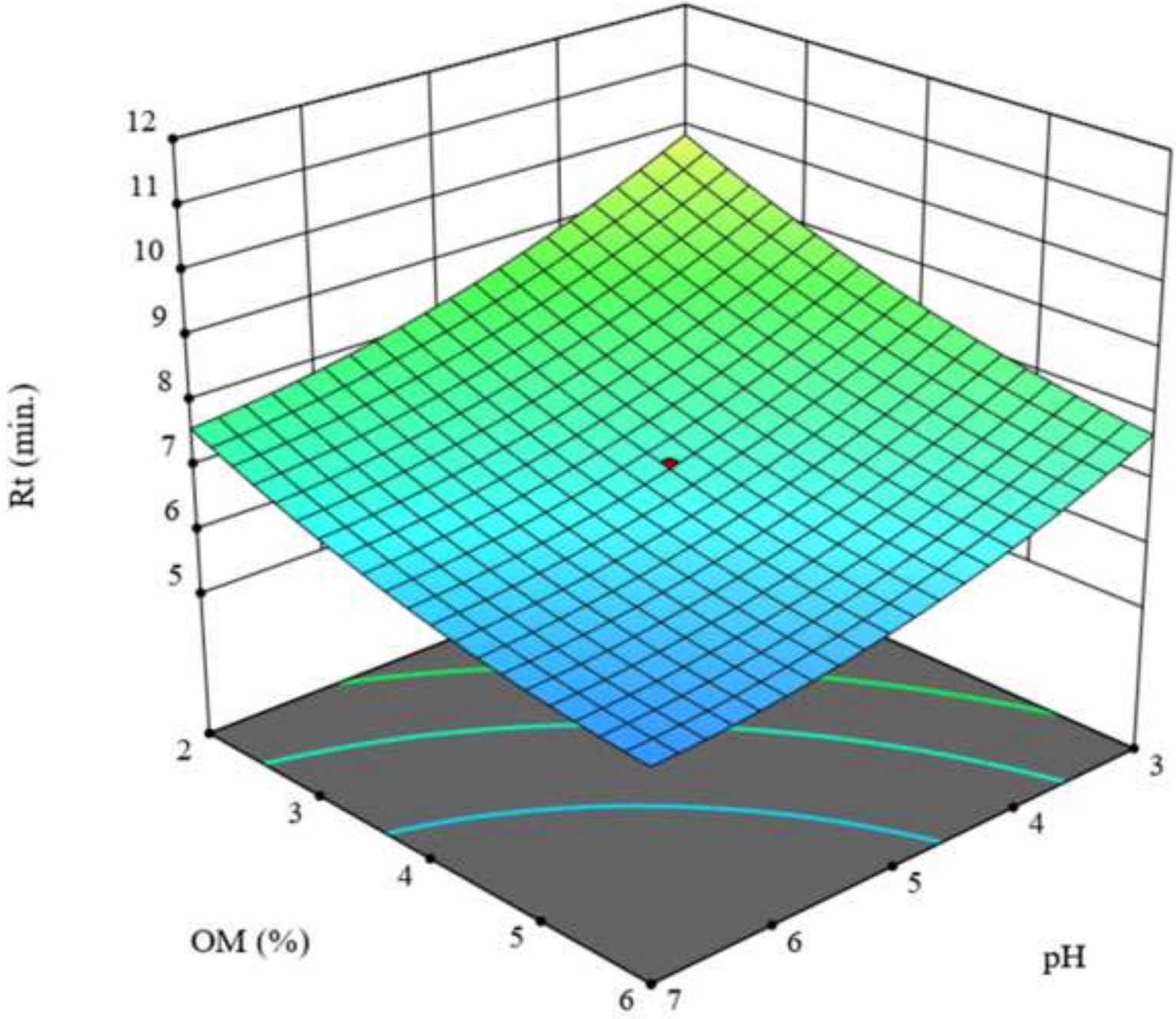

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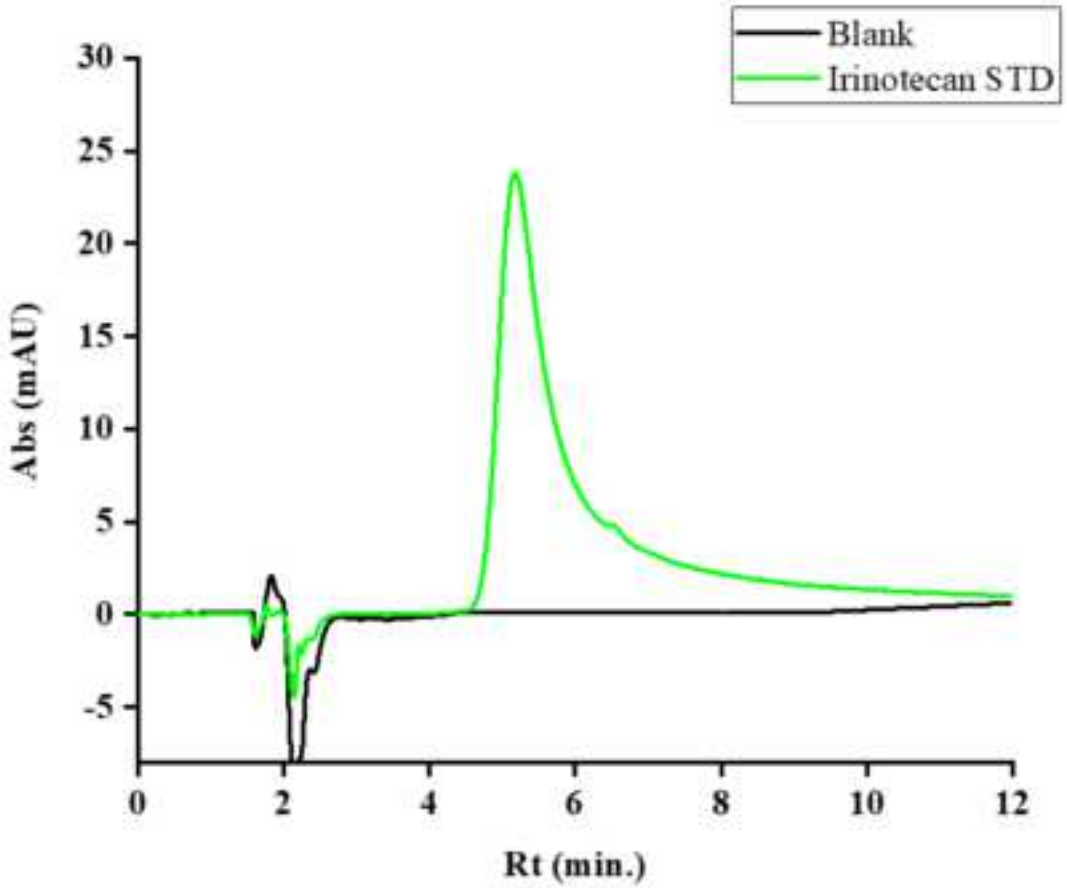

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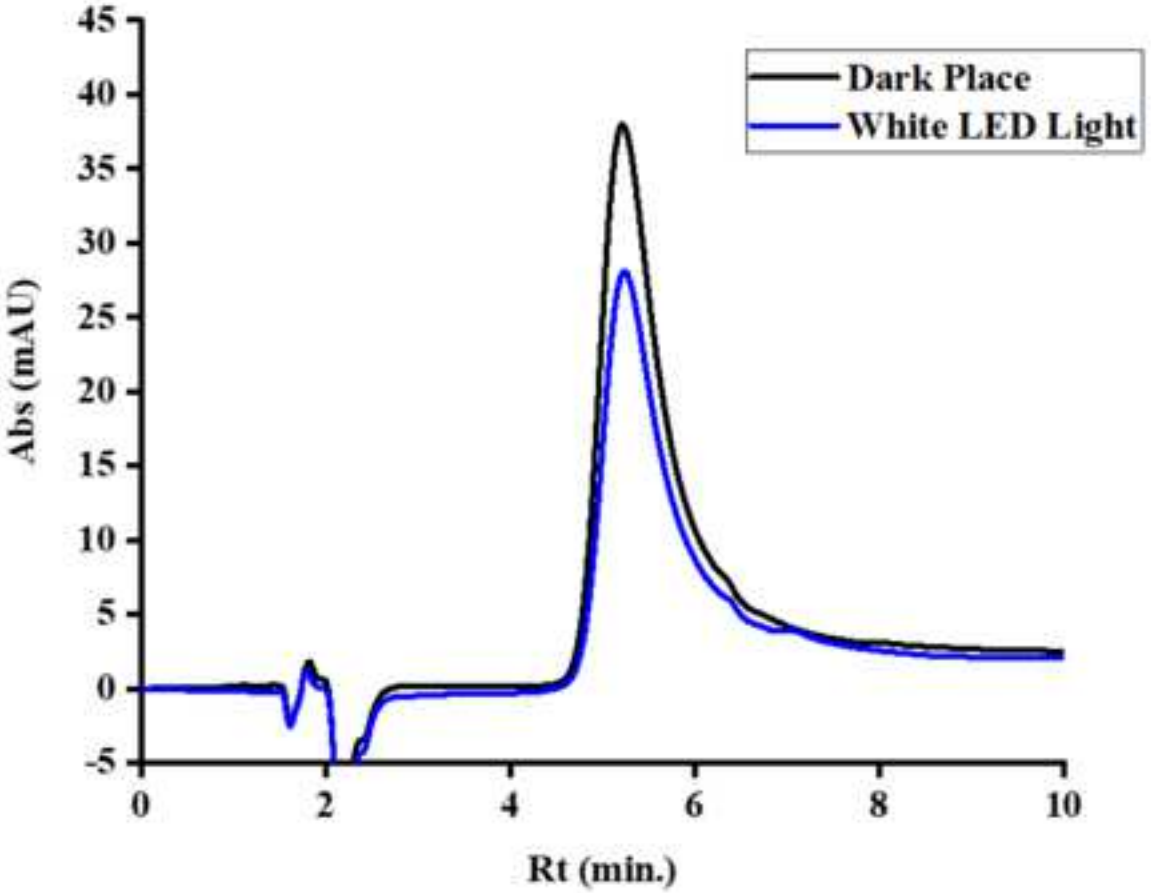

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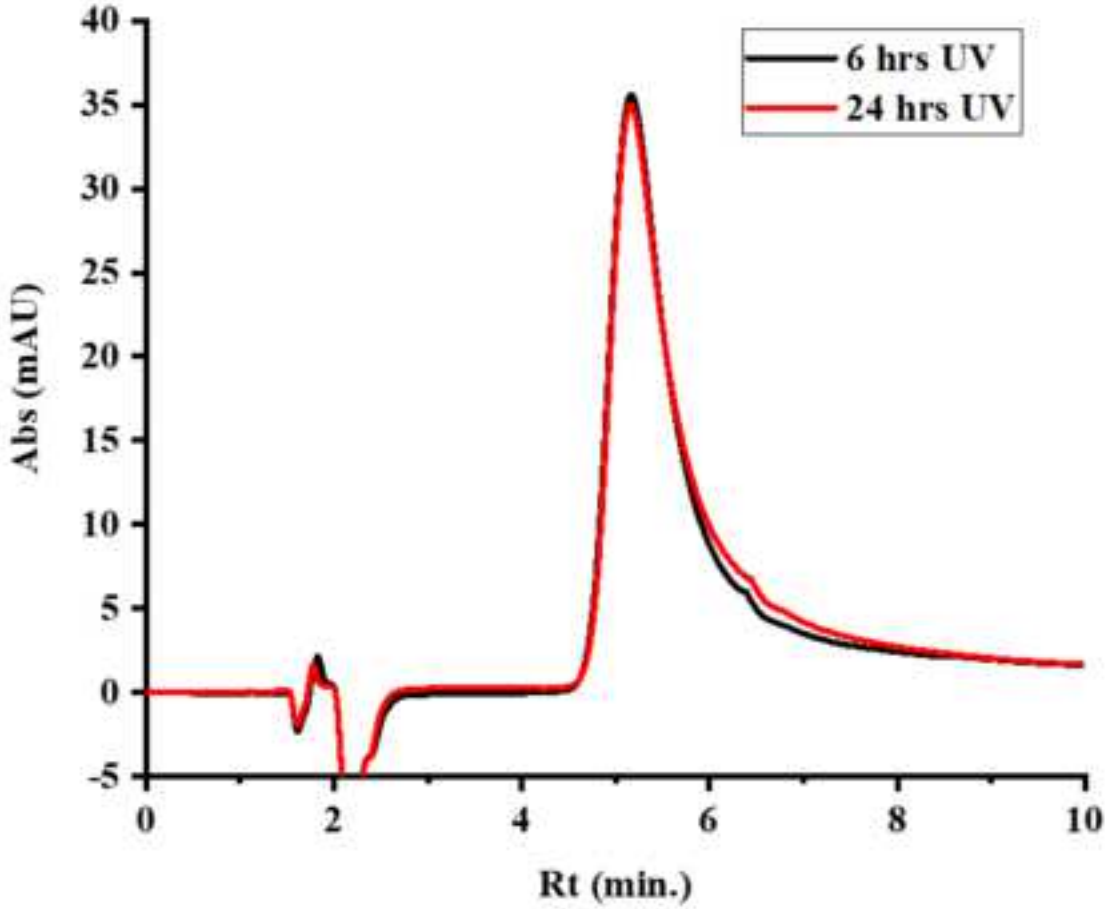
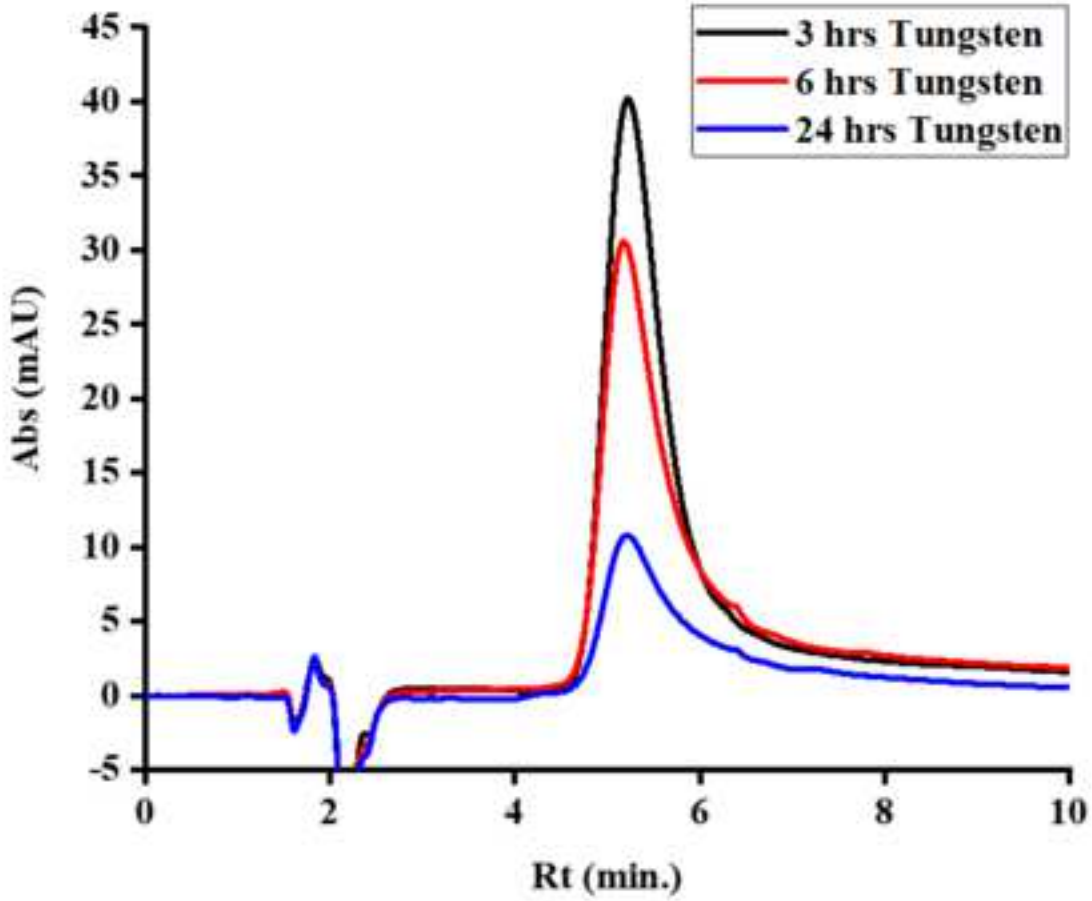


Figure 6



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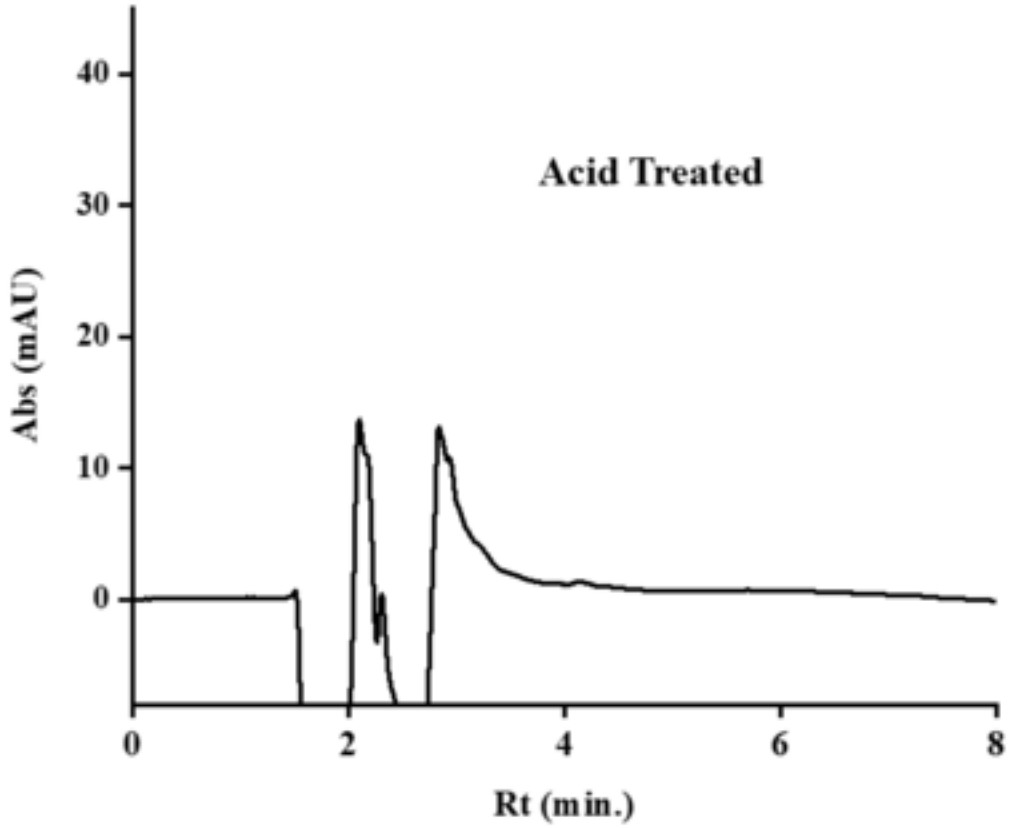

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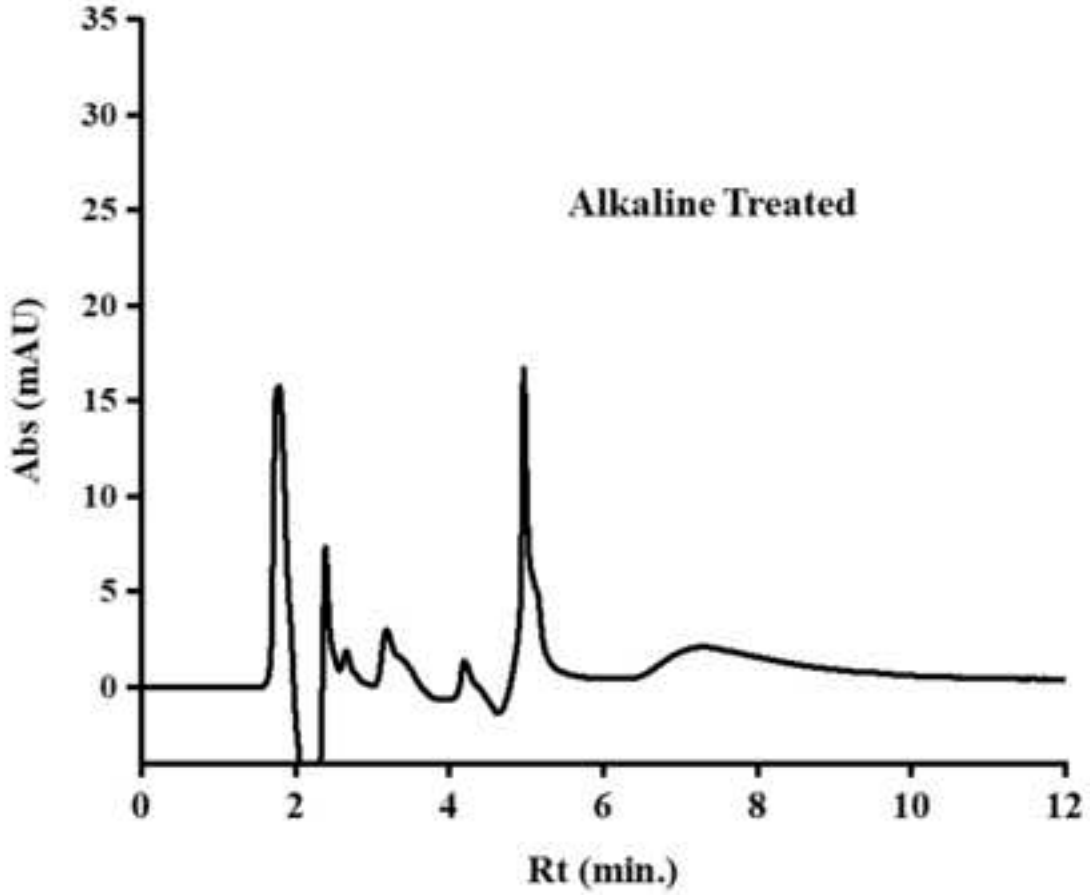


Figure 9

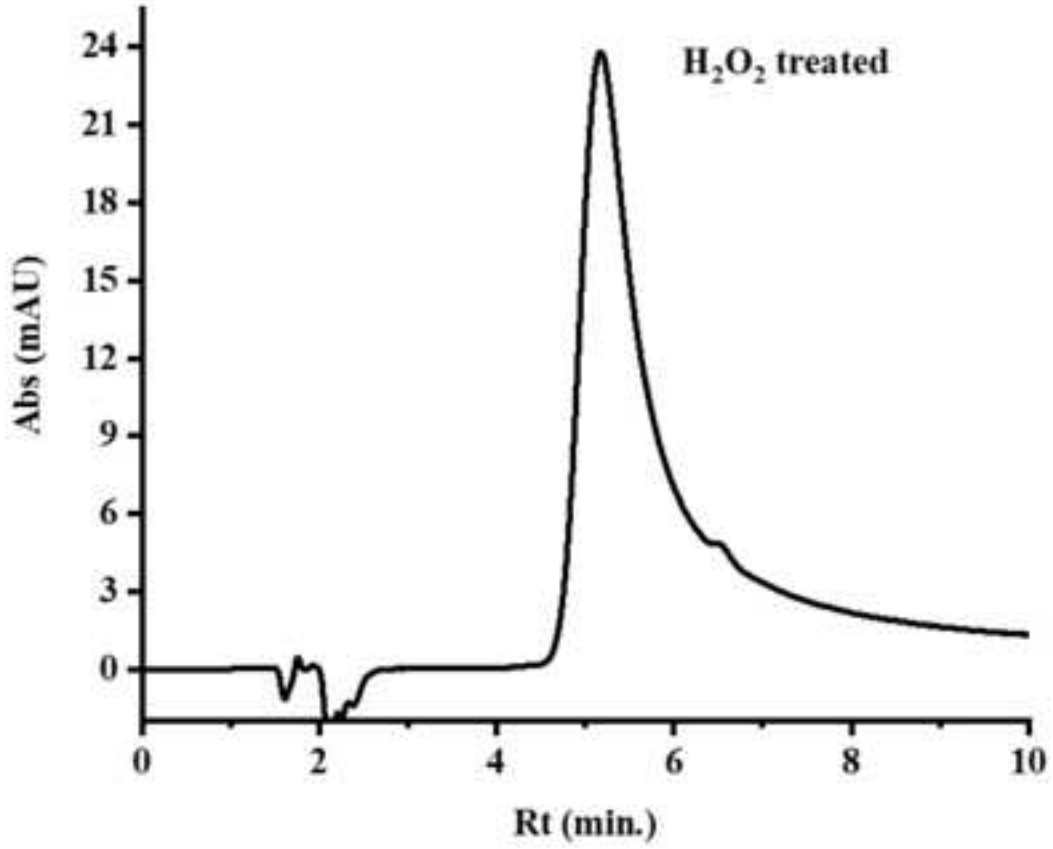


Table 1. Photolytic degradation condition of irrinotecan

Photocatalytic chamber	**Initial Temperature (°C)**	**Time interval (hours)**	**Final Temperature (°C)**
Dark chamber	25	24	25
Tungsten chamber (100W)	25	3	49
		6	60
		24	80
LED white light chamber (9W)	25	3	25
		6	25
		24	27
LED blue light chamber (5W)	25	3	27
		6	27
		24	30

Table 2. Central composite design for three- factor five-level in un-coded form

Central composite design (CCD) matrix and variables		**−1.68**	**−1**	**0**	**1**	**1.68**
X_1	SDS (M)	0.05	0.07	0.1	0.13	0.15
X_2	Organic Modifier (%)	1	2.5	4	5.5	7
X_3	pH	3	4	5	6	7

Table 3. Central composite design (CCD) matrix and results with five levels/three factors for optimization study of MLC_PDA method parameters

Dependent and independent variable for Response surface methodology (RSM)-central Composite Design (CCD) for three-factor five levels					
Exp. No	**X1 SDS conc. (M)**	**X2 Organic Modifier conc. (% Volume)**	**X3 pH of the Mobile phase**	**Response value**	
				Response value	**Predicted value**
1.	0.05	2	3	11.9	8.9
2.	0.15	2	3	8.9	9.2
3.	0.05	2	7	9.2	6.9
4.	0.15	2	7	6.9	9.2
5.	0.05	6	3	9.2	7.1
6.	0.15	6	3	7.1	7.4
7.	0.05	6	7	7.4	5.1
8.	0.15	6	7	5.1	10
9.	0.015	4	5	10	6.1
10.	0.184	4	5	6.1	9.7
11.	0.1	4	1.636	9.7	6.5
12.	0.1	4	8.363	6.5	9.4
13.	0.1	0.636	5	9.4	6.4
14.	0.1	7.363	5	6.4	7.1
15.	0.1	4	5	7.1	7.1
16.	0.1	4	5	7.1	7.1
17.	0.1	4	5	7.1	7.1
18.	0.1	4	5	7.1	7.1
19.	0.1	4	5	7.1	7.1
20.	0.1	4	5	7.1	8.9

Table 4. Test for validation of the experimental design model for response value Rt

Source	**DF**	**Adj. SS**	**Adj. MS**	**F-Value**	**P-Value**
Model	9	50.91	5.66	215.50	< 0.0001
SDS	1	19.36	19.36	737.45	< 0.0001
pH	1	14.11	14.11	537.56	< 0.0001
OM	1	12.65	12.65	482.05	< 0.0001
SDS×pH	1	0.0313	0.0313	1.19	0.0300
SDS×OM	1	0.1012	0.1012	3.86	0.0779
pH×OM	1	0.1013	0.1013	3.86	0.0779
SDS^2	1	1.92	1.92	73.33	< 0.0001
pH^2	1	2.12	2.12	80.60	< 0.0001
OM^2	1	1.41	1.41	53.59	< 0.0001
Residual	0.2625	10	0.0262		
Lack of Fit	0.2625	5	0.0525		
Pure Error	0.0000	5	0.0000		
Total	51.17	19			
S	**R^2(adj.)**	**R^2(pred.)**			
55.33	0.990	0.958			

Table 5: Analytical performance of the proposed method for doxorubicin

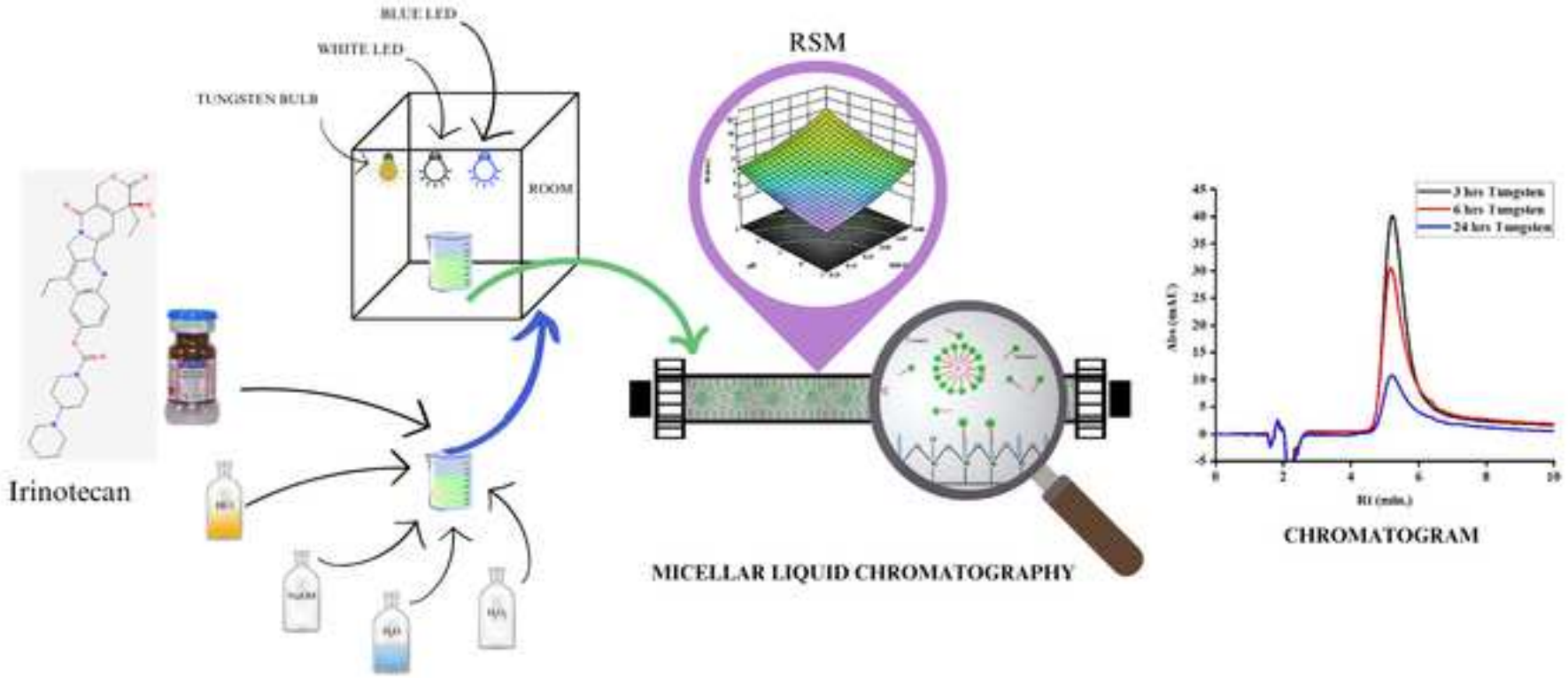
S.No.	Parameters	Results
1.	**Slope**	2.5987
2.	**Intercept**	0.0876
3.	**r**	0.999
4.	**Linear range (µg/mL)**	0.25-30
5.	**RSD% (n=5)**	5.1%
6.	**LOD (µg/mL)**	0.20
7.	**LOQ (µg/mL)**	0.25

Table 6: Precision (n=5) and accuracy (n=4) of the developed method for irinotecan.

Analyte	Added conc. (µg/mL)	Intra-day			Inter-day		
		Found (Mean ± SD)	Accuracy (ε_R, %)	Precision (RSD, %)	Found (Mean ± SD)	Accuracy (ε_R, %)	Precision (RSD, %)
Irinotecan	1	1.09±0.01	+9	1.5	1.05±0.06	+5	6.03
	2.5	2.56±0.02	+2.4	0.8	2.61±0.04	+11	1.63
	5	5.06±0.08	+6	1.5	5.04±0.07	+4	1.54

Graphical Abstract (for review)

Click here to access/download;Table (Editable version);itn.png



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: