Analytical Quality by Design (AQBD) Assisted Development and Validation of HPTLC Method for Estimation of Rottlerin in Topical Patch Formulation

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ABSTRACT

Background: Rottlerin, a natural polyphenolic ketone is a major constituent of Mallotus philippensis (Lam.) Mull. Arg. plant. Although, less explored it has diverse array of therapeutic applications. Objectives: The current work aimed to develop novel, simple, speedy, accurate, potent High Performance Thin Layer Chromatography Method (HPTLC) to separate, and quantify rottlerin from the herbal topical patch In-House formulation. Materials and Methods: This was the foremost attempt to develop and validate HPTLC method using Analytical Quality by design (AQbD) approach. Box Behnken design (BBD) coupled with response surface methodology approach was followed for the systematic optimization. The end results of toluene content, chamber conditioning time, and solvent front on Rf value of rottlerin was studied. The optimised method was validated as per ICH guidelines. Results: For HPTLC, stationary phase used was aluminium plates 60F254, coated with silica gel as. AQbD optimised conditions were mobile phase proportion namely toluene: ethyl acetate: methanol: ammonia (5:4:2:0.2), development distance 80 mm, band width 6mm, and chamber saturation time 10 min. The method generated well resolved and compact bands at Rf of 0.41±0.05. The developed method exhibited high accuracy, precision, robustness, and recovery. The quantity of Rottlerin in developed herbal patch was found to be 134.8 ng/band. Conclusion: In a nutshell, the present study undeniably vouches for a QbD based method development approach for Rottlerin and can be extrapolated to estimate it in other herbal extracts, or marketed formulations.

Keywords: Rottlerin, Analytical Quality by design (AQbD), HPTLC, Mallotus philippensis (Lam.) Mull. Arg., Topical patch.

INTRODUCTION

Mallotus philippensis (Lam.) Mull. Arg. commonly known as Kamala tree, is one of the well-known but less explored plants in traditional systems of medicines. It is very well described in ancient Indian literature Charaka Samhita and Sushruta Samhita. The fruits of the plant are most common element of various Ayurvedic and Unani formulations. It has wide array of therapeutic applicability such as Dermatological disorders,[2] Digestive disorders,[3,4] Helminthic disorders,[5,6] Inflammatory disorders,[7,8] Microbial infections,[9,10] Psychological disorders,[11] Reproductive disorders,[12] Respiratory disorders,[13] in vitro cytotoxicity,[14] etc.

Rottlerin, a natural polyphenolic ketone is the main bioactive component present in pericarp of Kamala plant. It is a 5,7 dihydroxy–2,2–dimethyl–6–(2,4,6 trihydroxy-3methyl-5acetyl benzyl)-8- cinnmoyl–1,2– chromene as displayed in Figure 1, also known as mallotoxin. Recent scientific data demonstrated the potential of rottlerin to modulate variety of molecular targets such as protein kinase C (PKC),5'- AMP activated protein kinase, p38 regulated kinase, C AMP dependent kinase, Casein kinase II, Glycogen synthase kinase 3 beta, AKT/PKB, and calmodulin–dependent kinase etc.[15] Also it can modulate Human Interleukin–6 (IL 6), Interleukin–1 beta (IL-1β), NF–Kappa B (NF-xB), transforming growth factor–beta type I receptor (TGF1 -β),Insulin–like Growth Factor Receptor (IGF 1R), Fibroblast Growth Factor–1 (FGF–1), Matrix Metalloproteinase (MMP 9).[16] Due to diverse therapeutic pertinence, rottlerin represents promising drug candidature to treat various diseases. But it lacks scientific evidence due to unavailability of precise analytical method to ensure safety, efficacy and quality of rottlerin, and its formulations. As per literature analytical approaches for

DOI:10.5530/pres.15.2.029

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determination of Rottlerin in plant extract, formulations, phytopharmaceuticals, and in biological fluids are scarce.

HPTLC is the ideal method of analysis due to its high speed, low cost, less mobile phase consumption, ease of sample detection, and quantitation, and less sample cleanup.\(^{[17]}\)

A QbD is the systematic approach for development and validation of analytical procedures. It offers better understanding and control of pharmaceutical process to develop superior products.\(^{[18]}\) This approach offers variety of designs to screen and optimised process variables that can influence responses. A QbD approach is a simple, fast, economic, and robust method development strategy.\(^{[19]}\) Many of the researchers have demonstrated utility of a Quality by Design (QbD) approach for HPTLC method development and estimation of analytes in various dosage forms as well as bioanalytical samples.\(^{[20-26]}\)

Till date, this approach has not been utilised for the analysis of Rottlerin as per our knowledge. Hence, the current work represents foremost attempts to develop simple, sensitive, precise, robust HPTLC method to analyse Rottlerin in herbal topical patch in formulation (In house) by applying design of experiment approach.

**MATERIALS AND METHODS**

**Instrumentation**

Initially TLC plates were splashed with Methanol and activated by heating at 110°C for 10 min. The development was carried out using 20 cm x 10cm and 10 cm x 10 cm, twin trough glass chamber (CAMAG, Switzerland) by ascending mode CAMAG HPTLC system consisting of CAMAG Linomat 5 sample applicator, Hamilton syringe (100µL), CAMAGE TLC scanner 3 with win CATS software version 1.3.0 and ultrasonic sonicator by Wensor was used for analysis. Design Expert \(^{\text{®}}\) version 13.0.11.0 (Stat–Ease Inc. Minneapolis) was employed for HPTLC method development and analysis.

**Materials and reagents**

Rottlerin was purchased from Life technologies (India) Pvt Ltd, New Delhi. The stationary phase was Silica gel 60F\(_{254}\) TLC plates (20 cm x 20 cm, 0.2 mm layer thickness, E Merck Ltd, Mumbai). Analytical grade chemicals and reagents (Merck Pharmaceuticals, India) were used in house prepared topical patch formulation was used for analysis.

**Preparation of Extract and topical hydrogel film**

The fruits of *Mallotus philippensis* (Lam.) Mull. Arg. were collected from Baneshwar village, Pune district, Maharashtra during flowering period of plant (January). The plant was authenticated from Jawaharlal Nehru Botanical Garden, Erandwane (Voucher No.1442). The fruits were dried. Red coloured powder present on glandular hairs of fruits was collected by tapping and brushing. The extract was prepared by macerating red powder with 90% ethanol for 48 hr. The extract was strained and concentrated by rotary vacuum evaporator. The dried extract was stored in air tight container and was used for topical hydrogel patch formulation.

The topical hydrogel patch was formulated by mixing 1% w/v of *Mallotus philippensis* (Lam.) Mull. Arg. extract with 8:2 ratio of 10% w/v of Polyvinyl alcohol (PVA) and 2% w/v of Chitosan containing 0.3% w/v of zinc sulphate and 5% w/v of Polyethylene glycol PEG 400. The blend was continuously stirred to get a uniform solution. It was poured in petri dish lubricated with glycerin. It was dried at room temperature for 24 hr to get uniform hydrogel films. This patch was further subjected to chromatographic evaluation.

**Chromatographic conditions**

On the precoated silica gel aluminium plates 60F\(_{254}\) samples were spotted using CAMAG microliter syringe as bands of 6 mm width with CAMAG Linomat 5 sample applicator (CAMAG, Switzerland). The mobile phase used was Toluene: Ethyl acetate: Methanol: Ammonia (5:4:2:0.2). The trial–and-error method was implemented to develop mobile phase and optimised by Box Behnken design (BBD). The chamber saturation time (Optimised with BBD) was 10 min and the development was carried out up to the length of 80 mm (Optimised with BBD). The plates were dried by an air dryer. The plates were scanned by CAMAGE TLC scanner 3 using win CATS software version 1.3.0 at 298 nm.

**Preparation of Standard and Sample solution of formulation**

The precisely weighed 10 mg standard Rottlerin was taken into 10 mL volumetric flask. The drug was solubilized in 10 mL methanol with vigorous shaking and then volume was made up to obtain final concentration of 1 mg/mL. From this resultant solution 1 mL was taken and volume was made up with methanol to achieve 100 µg/mL final concentration.

The accurately weighed topical patch formulation equivalent to 10 mg of Rottlerin standard was transferred to another volumetric flask (10 mL). The volume was adjusted with HPLC grade methanol to obtain final concentration 1 mg/mL. The it was kept in sonicator for 15 min to extract drug from the patch.
From this resultant solution 1 mL was taken and volume was made up to 10 mL with HPLC grade methanol to achieve final concentration of 100 µg/mL.

Preparation of sample solution of extract: Accurately weighed 10 mg of Mallotus philippensis (Lam.) Mull. Arg. plant extract was dissolved in 100 mL of methanol to get final concentration of stock solution 100 µg/mL. Then it was strained using 0.45µ membrane filter and then used further for HPTLC.

**Development of Method by QbD approach**

For method development of rottlerin initially the analytical target profile (ATP) was defined. The Toluene content, saturation time, and development distance were selected as critical method parameter (CMPs). The retention factor was identified as CAA. The optimization was carried out using Box Behnken design (BBD) of three factors and three levels. With help of Design Expert version 13.01.11.0 (Stat–Ease Inc. Minneapolis) seventeen runs were conducted. Response surface methodology was used to find out relationship between dependent and independent variables. The suitability of model was estimated considering $R^2$ value, Standard Deviation (SD), Adequate precision, and coefficient of variance (%CV). Variance analysis (ANOVA) was employed for validation of significance of model. The Lack of Fit Test, $p$ value, and Model F value were estimated to test the significance of model. For polynomial equation, model coefficient of statistical significance $< 0.05$ was considered. The preliminary experimentation were performed to select dependent and independent variables. The low, medium, and high levels of variables have been displayed in Table 1. For response surface analysis 3D response surface plots were considered. Table 2 explains design matrix of BBD which accounts for 17 runs.

ICH guidelines were followed for validation of method. The parameters such as Linearity, Precision, Limit of Detection (LOD), Limit of Quantification (LOQ), recovery, and robustness were studied.
**Calibration curve of rottlerin**

Rottlerin was dissolved in Methanol to prepare stock solution (100 µg/mL). The stock solution was plotted in different volumes namely 1 to 5µL on TLC plates in triplicates to achieve final concentration of 0.1 to 0.5 µg/ spot of rottlerin. The graph of peak area verses drug concentration was plotted and analysed by Linear Least Square Regression.

**Linearity**

Linearity of developed method for estimation of rottlerin was analysed using various concentrations of standard stock solution ranging from 100 to 500 ng/band. The results were plotted as peak area against band concentration.

**Precision**

The precision study was demonstrated by repeating the analysis of sample at different time interval within the same day (Intraday precision) and on the next day (Inter-day precision) and % RSD was determined.[28]

**Accuracy**

The recovery of sample at three different levels (80,100,120% addition) was performed to cheque Accuracy. The sample was spiked with known amount of standard, analysed and % RSD was calculated.[29]

**LOD and LOQ**

Limit of detection (LOD) means of method of analysis means the lowest detectable amount of analyte but not necessarily exactly quantifiable. It was determined by formula:

\[ \text{LOD} = 3.3 \times \text{Standard Deviation (SD) of response/ calibration curve slope} \]

Limit of Quantification (LOQ) means lowest quantifiable amount of sample with suitable precision and accuracy. It can be estimated using equation

\[ \text{LOQ} = 10 \times \text{Standard Deviation (SD) of response/ calibration curve slope} \]

**Robustness**

It was determined to cheque reliability of method. To cheque robustness of method slight changes were deliberately made in mobile phase composition and saturation time. The effect of these changes on retardation factor was determined.

**RESULTS**

**Optimization of chromatographic conditions using QbD and statistical analysis**

For determination of rottlerin by HPTLC method preliminary studies were carried out using various combinations of solvents mainly toluene, methanol, ethyl acetate, formic acid, dichloromethane, and ammonia. The chromatograms were recorded at 298 nm. The aim for optimization process was to obtain Rf value between 0.2 to 0.8. The preliminary trials indicated toluene concentration, development distance, and chamber saturation have significant effect on Rf value in HPTLC. So, these factors were selected for method optimization by DOE. The final optimised chromatographic conditions were mobile phase ratio namely Toluene: Ethyl acetate: Methanol: Ammonia (5:4:2:0.2), band width 6mm, Development distance 80 mm, and chamber saturation time 10 min. It gave highest resolution and Rf value of 0.41. The effect of CMPs on method CAA were indicated by half Normal plot (Figure 2).

The results of regression analysis with low standard deviation value 0.012 and higher R square value 0.9949 designated excellent adequacy of regression model. The coefficient of variation (%CV) was 2.97 which was less than 10% projected reproducibility of model. Adequate precision dictates signal to noise ratio. It should be greater than 4. As per fit statistics the result of adequate precision was found to be 41.40 dictating adequacy of signal. So, this model could assist in design space navigation.

Seventeen runs for design were randomly conducted to minimise bias. The polynomial equation obtained for Rf value was as follows:

\[ Y = 0.4100 + 0.0825A + 0.0175B - 0.0475C - 0.0750AB + 0.0075AC -0.0025BC +0.0400A^2 -0.0350B^2 + 0.0275C^2 + 0.1025AB -0.0225AB^2 (1) \]

Where, Y = Rf values, A = Toluene content, B= Saturation Time, C = Development distance

As per equation it was clear that toluene concentration (factor A) and Saturation time (factor B) positively affected the Rf value and Development distance (factor C) had negative effect.

The model validation was carried out by analysis of variance (ANOVA). As per ANOVA (Table 3), the probability value was
**Figure 3:** Response surface 3D plots showing interaction between Toluene concentration (A) vs Saturation time (B).

**Figure 4:** Response surface 3D plots showing interaction between Toluene content (A) vs Development distance (C).
found to be < 0.0001, hence the model significantly (p<0.05) predicted R\textsubscript{f} value. It implied due to noise merely a 0.01% chance is there for large F–value. The the model was found to be significant because of model F–value of 88.54. For response R\textsubscript{f} value, all three factors like toluene concentration (factor A), Saturation time (factor B), and Development distance (factor C) were found to be statistically significant (p<0.05).

To study effect of these factors on response R\textsubscript{f} value, 3 D response surface plots (Figures 3, 4, 5) were also analysed.

As depicted by the graphs increase in R\textsubscript{f} value was directly proportional to increase in toluene content and saturation time. On the contrary, increase in development distance decreased R\textsubscript{f} value. The curve lines in these plots indicated nonlinear relation between factors and R\textsubscript{f} values.

**Figure 5:** Response surface 3D plots showing interaction between Saturation time (B) vs Development distance (C).
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Figure 6: Perturbation plots showing effects of factors on R<sub>f</sub> value of Rottlerin. (A: Toluene content, B: Saturation time, C: Development distance).

Figure 7: Desirability plots showing effects of factors on R<sub>f</sub> value of Rottlerin.

Figure 8: Overlay plots showing effects of factors on R<sub>f</sub> value of Rottlerin.

Figure 9: HPTLC chromatogram of Rottlerin R<sub>f</sub> 0.41±0.05

Figure 10: Calibration curve for Rottlerin (100 to 500 ng/band).

Table 4: Linear Regression data for estimation of Rottlerin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rottlerin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range ng/spot</td>
<td>100–500</td>
</tr>
<tr>
<td>Slope</td>
<td>30.003</td>
</tr>
<tr>
<td>Intercept</td>
<td>7174.41</td>
</tr>
<tr>
<td>Correlation coefficient (r&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.9949</td>
</tr>
</tbody>
</table>
Table 5: Results of Accuracy study.

<table>
<thead>
<tr>
<th>% Recovery level</th>
<th>Initial Amount present ng/spot</th>
<th>Amount Added ng/spot</th>
<th>Total amount expected ng/spot</th>
<th>Amount recovered ng/spot</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>300</td>
<td>240</td>
<td>540</td>
<td>538</td>
<td>99.62</td>
</tr>
<tr>
<td>100</td>
<td>300</td>
<td>300</td>
<td>600</td>
<td>602</td>
<td>100.33</td>
</tr>
<tr>
<td>120</td>
<td>300</td>
<td>360</td>
<td>660</td>
<td>670</td>
<td>101.51</td>
</tr>
</tbody>
</table>

Table 6: Results of Intraday precision study.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Mean Peak area</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning</td>
<td>16611.25</td>
<td>48.87</td>
<td>0.29</td>
</tr>
<tr>
<td>Afternoon</td>
<td>15820.25</td>
<td>69.66</td>
<td>0.44</td>
</tr>
<tr>
<td>Evening</td>
<td>15305.5</td>
<td>58.47</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Table 7: Results of Inter day precision study.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Mean Peak area</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>16611.25</td>
<td>48.87</td>
<td>0.29</td>
</tr>
<tr>
<td>Day 2</td>
<td>14609.5</td>
<td>71.84</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Table 8: LOD and LOQ data for Rottlerin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>LOD (ng/spot)</th>
<th>LOQ (ng/spot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rottlerin</td>
<td>43.32</td>
<td>131.30</td>
</tr>
</tbody>
</table>

Table 9: Results for Robustness experiments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Altered Conditions</th>
<th>Effect on Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturation Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>Original</td>
<td>10 min</td>
<td>0.33</td>
</tr>
<tr>
<td>15 min</td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>Mobile Phase composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T: EA: M: A (4:5:2:0.2)</td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>Original</td>
<td>T: EA: M: A (5:4:2:0.2)</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>T: EA: M: A (6:3:2:0.2)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 10: Quantification of Rottlerin in extract and topical patch formulation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rottlerin content (ng/ band)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallotus philippensis fruit extract</td>
<td>158.0</td>
</tr>
<tr>
<td>Topical patch formulation</td>
<td>134.8</td>
</tr>
</tbody>
</table>

The perturbation plots (Figure 6) were also studied to analyse effect of each factor on Rf value. At reference point when all factors were constant, the plots revealed changes in the response, the curvature of line designates sensitivity to specific factor. The Rf value was increased with increase toluene content and decrease in development distance. There was no significant increase in Rf value with increase in chamber saturation time.

To achieve best chromatographic performance numerical optimization technique by desirability approach was used. The desirability function ranges from undesired response (0) to fully desired response (1). The desirability value close to 1 dictate global optimization of selected combination of different criteria. Desirability and Overlay plots are displayed in Figure 7 and
Figure 8 respectively. All the constrains are satisfied in the yellow area in overlay plot.

**Validation of proposed Method**

The DOE optimised chromatography conditions such as mobile phase proportion namely Toluene: Ethyl acetate: Methanol: Ammonia (5:4:2:0.2), development distance 80 mm, band width 6mm, and chamber saturation time 10 min efficiently resolved rottlerin at $R_f$ value 0.41±0.05 (Figure 9).

HPTLC method developed for estimation of rottlerin exhibited good linear correlation between peak area and applied concentration, ranges from 100 to 500 ng/band (Figure 10). As depicted in Table 4, the value of correlation coefficient was observed to be 0.9949. The peak area (y) was found to be proportional to concentration of rottlerin following regression equation $Y= 30.003x + 7174.41$.

**Accuracy**

Accuracy was reported as the percent recovery by addition of known concentration of analyte in the sample. It was performed in triplicates by addition of known amount of standard solution at three different concentration levels namely 80, 100, and 120%. As displayed in Table 5 the percent recovery was observed to be within acceptable limits (98 to 102%). Hence the method was found be accurate.

**Precision**

Precision calculated as percentage Relative Standard Deviation (%RSD). As displayed (Table 6 and 7 respectively) percent RSD of peak areas obtained for intraday and inter day variation was within acceptable criteria than is less than 2%. Hence the method was precise.

**LOD and LOQ**

LOD and LOQ was determined considering Standard Deviation of responses and mean calibration curve slope. The results displayed (Table 8) indicated sensitivity of the projected method.

**Robustness**

As per the results depicted in Table 9, slight changes in $R_f$ values were observed but no alterations on peak resolution was noted, hence it can be concluded that the method was robust.

**Quantification of Rottlerin in extract and topical patch formulation**

The amount of Rottlerin in extract and formulation was observed to be 158 ng/band and 134.8 ng/band respectively (Table 10).

**DISCUSSION**

A QbD approach is a simple, fast, economic and robust for development, and validation of analytical methods. The present work successfully attempted In-house herbal patch formulation using hydroalcoholic extract of *Mallotus philippensis* Lam. Mull. Arg. (MP) plant. For estimation of rottlerin Box Behnken design (BBD) with response surface methodology was implemented at three factors and three levels to understand the factor and response relationship. Based on the results of 3D response surface plots and perturbation plots, toluene concentration (A), and development distance (C) had significant effect on $R_f$ value as compared to saturation time (B). The DOE optimised HPTLC conditions offered well resolved peaks of rottlerin at $R_f$ value 0.41±0.05. The current method of HPTLC was found to be accurate, precise, sensitive, and robust.

**CONCLUSION**

A foremost attempt successfully developed modest, speedy, accurate, precise, and robust HPTLC method for detection of rottlerin in *Mallotus philippensis* (Lam.) Mull. extract and its topical patch in-house formulation. Box Behnken design (BBD) coupled with response surface methodology approach was followed for optimization of chromatographic conditions. The experimental design described scrutiny of key components such as toluene content, saturation time, and development distance. The interactions were analysed and chromatographic conditions were optimised. ICH guidelines were used to validate HPTLC method. In a nutshell, the present study undeniably vouches for a QbD based method development approach for Rottlerin and can be extrapolated to estimate it in other herbal extracts, or marketed formulations.

**ACKNOWLEDGEMENT**

The authors are thankful to Principal, BVDU’s Poona college of Pharmacy, Pune for providing necessary research facilities.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ABBREVIATIONS**

AQBD: Analytical Quality by Design; HPTLC: High Performance Thin Layer Chromatography; BBD: Box Behnken design; IL 6: Interleukininterleukin–6; IL-1β: Interleukininterleukin–1 beta; NF-κB: NF–kappa B; TGFβ1: Transforming growth factor–beta type I receptor; IGF 1R: Insulin–like Growth Factor Receptor; FGF–1: Fibroblast growth factor; MMP 9: Matrix Metalloproteinase. LOD: Limit of Detection; LOQ: Limit of Quantification; RSD: Relative standard deviation; DOE: Design of Experiments; % CV: coefficient of variance; ANOVA: Analysis of Variance; SD: Standard Deviation; ATP: analytical...
target profile; CMPs: Critical method parameter, CAA: Critical analytical attribute.

SUMMARY

The major constituent of *Mallotus philippensis* Lam. Mull. Arg. (MP) plant, rottlerin is less explored but has wide therapeutic utility. In the current work, In- house herbal patch formulation was fabricated using hydroalcoholic extract, and analysed by HPTLC. In view of rottlerin estimation, this was the foremost attempt of Analytical Quality by design (AQbD) based HPTLC method development, and validation. The quantification of rottlerin was carried out on precoated silica gel aluminium plates 60F254 with mobile phase Toluene: Ethyl acetate: Methanol: Ammonia (5:4:2.0:2.), chamber saturation time of 10 min, and the development distance of 80 mm. The conditions were optimised with Box Behnken design (BBD). The well resolved and compact bands of rottlerin were observed at Rf of 0.41±0.05. The amount of Rottlerin found in extract and In- house patch formulation was 158 ng/band and 134.8 ng/band respectively. This Analytical Quality by Design (AQbD) Assisted HPTLC method exhibited high accuracy, precision, robustness, and recovery. Hence, the present work could be extrapolated to estimate rottlerin in herbal extract, and formulations.

REFERENCES