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Original Article

# DEVELOPMENT VALIDATION OF QUERCETIN COMPOUNDS USING RP-HPLC AND *IN VITRO* ACTIVITY STUDIES ON *MELASTOMA MALABATHRICUM* LEAF NANOCREAM FOUNDATION PREPARATIONS

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**ABSTRACT**

**Objective:** A simple, accurate, precise, sensitive, and robust analytical method has been developed to analyze quercetin in the nano-cream foundation formula added to the ethyl acetate fraction of *Melastoma malabathricum* leaves.

**Methods:** The validation is consistent with ICH guidelines using a reversed-phase HPLC method with mobile-phase acetonitrile-phosphoric buffer conditions at pH 5.51 (40:60 v/v). The stationary phase has a C-18 column, a 0.5 ml/min flow rate, and an Ultraviolet detector at wavelength 370 nm. Furthermore, antioxidant activity testing used the FRAP method, and the SPF value was measured using a Elisa reader with wavelength 615nm and UV/Vis spectrophotometer in the wavelength range of 290-390 nm.

**Results:** The results showed that the retention time of quercetin was 11.462 min with a tailing factor of 1.045. Regression equation y=170220x-85497 had a concentration range of 3-18 µg/ml and a correlation value of 0.9998. The limit of Detection (LOD) and Limit of Quantification (LOQ) were 0.236 and 0.786 µg/ml, respectively. The accuracy method for the recovery value at concentration levels of 80%, 100%, and 120% was 95.233%-109.446%. The repeatability of intra-day and inter-day variations express the %RSD values of 1.258-1.378% and 1.012-1.258%. Meanwhile, the method's strength by changing the column temperature, maximum wavelength, mobile phase composition, and the flow rate was expressed by the %RSD value of 0.045-0.067%, 0.069-1.642%, 0.107-0.165%, and 0.141-0.318%, respectively. The analysis results of the quercetin content in the nano cream foundation formula were 0.021728±5.83x$10^{-4}$ mg/g. Antioxidant activity with an $IC_{50}$ value was 1444.157±15.538 µg/ml, and the SPF at a concentration of 8000 µg/ml was 11.370±1.083.

**Conclusion:** The development method of quercetin is simple, accurate, precise, sensitive, and robust. Quantitative analysis of quercetin biomarkers in nano cream preparations containing the ethyl acetate fraction of *Melastoma malabathricum* leaves using the RP-HPLC method.

**Keywords:** Nanocream foundation, Validation, Ethyl acetate fraction, *Melastoma malabathricum*


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## INTRODUCTION

Flavonoids are phenolic compounds found in secondary metabolites of plants, and their antioxidant properties are known to have health benefits. Previous studies have identified more than 4000 flavonoid compounds [1]. Meanwhile, quercetin is a compound belonging to the flavonol subclass, and 3,5,7,3',4'-pentahydroxyflavone is an antioxidant found in fruits and vegetables such as apples, grapes, lemons, tomatoes, onions, lettuce, broccoli, kale, and cottonseed. It is one of the higher compounds, including *Ginkgo Biloba*, *Apocinum Venetum*, *Poacinum Hendersoni Fiklindica*, *Olive Oil*, Propolis from *Beehives*, and other spices [2]. In the flavonol subclass, quercetin has an o-hydroxy structure on ring B, a 2,3 double bond attached to a 4-oxo function on ring C, and oxo functions on the four rounds A and C. The antioxidant functions can be protected by glutathione peroxidase, reductase, catalase, and superoxide dismutase [3].

The photoprotective mechanism of quercetin is transformed through heat, light, or degradation of the compound by absorbing ultraviolet energy. The topical application effectively prevents liposome peroxidation induced by UVC radiation [3]. Studies by (Yin *et al.*, 2013) found that topical formulations of the compound applied to the skin of experimental animals successfully inhibited damage caused by UVB rays [4]. Furthermore, He *et al.*, 2021 stated that creams containing quercetin routine had the same 10% SPF level as homosalate and titanium dioxide [5].

According to a study by Jofry *et al.*, 2012, *Melastoma malabathricum* contains phenolic compounds, such as kaempferol, quercetin, and rutin. Analysis with Gas Chromatography by Apridamayanti *et al.*, 2022 is known that the n-hexane fraction contains a diterpene group compound, and the chloroform fraction contains fatty acid, where the predominant compound are 2-Pentadecanone 6-10-14-trimethyl (11.75%) and 9-12-15-Octadecatrienoic acid, methyl ester (Z, Z, Z) (15.57%). A survey by Apridamayanti *et al.*, 2022[a] using RP-HPLC showed that the quercetin content in the ethyl acetate fraction was 0.93±0.06 mg/g, with antioxidant activity of 1.9±0.12 ppm (DPPH). The antioxidant activity with the FRAP method is 4.2±1.5 ppm, while the SPF value was 59.3±0.9. Pratiwi *et al.*, 2021, found that nanospray formulated from SNEDDS has a synergistic combination of *Melastoma malabathricum* with gentamicin [6-9].

Nanotechnology is a branch of science that can make atoms and molecules into nano sizes of 1-100 nm [34]. It has been widely applied in various fields, namely pharmaceuticals, cosmetics, biomedical and drug delivery, defense and security, electronics, automobiles, aerospace, and metallurgy [10]. The entry is developed towards cosmetics through nanoparticles that carry particles and infiltrate well into the skin. The development of nanoemulsions, liposomes, and lipid nanoparticles has significant potential as a carrier of compounds that can improve skin layer repair [10, 33]. Meanwhile, cosmetic manufacturers use various nanomaterials in several skin cosmetic products, such as Cleansing agents, Moisturizing agents, Antiaging creams, and Exfoliators [10]. Nanoparticles have been developed in sunscreen products that protect the skin from UV rays and cancer. Nanophytomedicine created using zinc oxide and titanium oxide compounds is known to have protective activity against UV rays [11].

An essential aspect of quality control of products with natural ingredients is the analysis of chemical markers, which are chemically contained in plant products [12]. The analytical technique developed

can identify and measure chemical marker compounds. One of the analytical techniques used in creating these methods is using performance liquid chromatography (HPLC) instruments [13]. According to Seraglio *et al.*, 2016, GC-MS, LC-MS/MS, and HPLC are instruments used to analyze phenolic compounds as a marker in the formulation. RP-HPLC is also developed to analyze and validate the testing method for phenolic marker compounds [14]. Validation is a technique that provides documented evidence, where the analysis method used for the test follows its intended use. It involves a procedure demonstrating the method's reliability using parameters where linearity, accuracy, repeatability, selectivity, and sensitivity should be considered [15].

Tests related to analysis validation on nano cream preparations with adding the ethyl acetate fraction *Melastoma malabathricum* using quercetin as a marker compound should be performed. Quantitative analysis of marker compound is a follow-up to the application in manufacturing nano cream preparations with the potential as antiaging and photo protectors. In this study, the assay analysis technique and method validation using quercetin as a marker compound was carried out in the nano cream preparation with the ethyl acetate fraction of *Melastoma malabathricum* as an antiaging and photoprotector preparation. *In vitro*, antioxidant activity was studied using the FRAP method and measuring the SPF value on trial.

## MATERIALS AND METHODS

### Materials and excipients

Standard of Quercetin (Sigma-HPLC grade), Acetonitrile (Merck-HPLC grade), $NaH_2PO_4$ (Merck), $NaHPO_4$ (Merck), Double distilled water. Cetyl alcohol (bratachem), liquid paraffin (bratachem), propylene glycol (bratachem), stearic acid (bratachem), sodium lauryl sulfate (bratachem), propylparaben (bratachem), triethanolamine (bratachem), methylparaben (bratachem). pH meter (Hanna), Sonicator (Bronson), HPLC instrument (Shimadzu), Spectrophotometer UV/Vis (Shimadzu), Ellisa reader (Thermo).

### The instrumentation

HPLC analysis study using Shimadzu Corporation UFLC equipped with reservoir tray, Prominence Degasser (DGU-20A5), Prominence Liquid Chromatography (LC-20AD), Prominence Communication Bus Mobile (CBM-20A), Prominence UV Detector (SPD-20A), and Colom Oven (CTO-20 A).

### Preparation of mobile phase

In this study, mobile phase optimization used two combinations (45:55v/v) and (40:60v/v). Mixed buffer phosphate and acetonitrile were carried out using the gradient method in a ratio (45:55 v/v) and (40:60 v/v).

Buffer phosphoric pH 5.51 was prepared with $NaH_2PO_4$ and $Na_2HPO4$ solution with a ratio of 24:1 v/v.

### Nanocream preparation

Nanocream preparation in this study was made with a combination of cetyl alcohol, liquid paraffin, and propylene glycol. Liquid paraffin, cetyl alcohol, and stearic acid as lipid phase. Sodium lauryl sulfate, propylparaben, triethanolamine, methylparaben, propylene glycol, and aqua dest as a polar phase. The lipid phase and water phase were melted in different containers at 70 °C, then mixed in a hot mortar, crushed, and added ethyl acetate fraction of *Melastoma malabathricum L.* Next, the mixture was put in a Homogenizer at 750 rpm for 10 min.

### Validated analytical method

### Instrumentation RP-HPLC analysis of quercetin

The sample is a nano cream loaded ethyl acetate fraction of *Melastoma malabathricum* dissolved in acetonitrile. The fraction obtained from previous studies carried out the maceration process on *Melastoma malabathricum* leaves. It continued the separation process using n-hexane, chloroform, ethyl acetate, methanol, and water as solvents. The active compound added to the nano cream sample in this study is the ethyl acetate fraction of *Melastoma malabathricum***.** Furthermore, it was filtered using filter paper of 0.2 µm membrane (Whatman) and directly injected into the HPLC system. Quercetin as a standard stock solution was prepared by dissolving an accurately weighed amount of 25 mg in 25 ml acetonitrile as stock solution and made different as a calibration curve in the range of 3 µg/ml-18 µg/ml. The standard solutions were filtered through a 0.2 µm membrane filter (Whatman) before injection into the HPLC system [8].

### Chromatography condition in RP-HPLC

The chromatography condition in this study, according to (Apridamayanti *et al.*, 2022), with modification [8]. Colom chromatography used in this study is VP-ODS shim-pack C-18, 250x4.6 (mm), reverse phase column packed with 4.5 µm diameter particles, in optimization of mobile phase acetonitrile-buffer phosphoric with pH 5.51 (40:60 v/v) and (45:55 v/v). The mobile phase was filtered through a 0.2 µm membrane and de-aerated ultrasonically before use—the temperature in this study at 30 °C. Volume injection is 30 µl, and flow rate is 0.25, 0.5, 0.75, and 1 ml/min—the maximum wavelength with UV detector for quercetin at 370 nm.

### Assay validation

The International Council validated the development of the RP-HPLC method for quantifying quercetin for Harmonisation (ICH). The validation in this study concerns linearity, range, precision, accuracy, sensitivity, and robustness [16].

### Linearity and range

Different standard solutions were prepared to evaluate the method's linearity and range by diluting the standard stock solution with a mobile phase in the acetonitrile range of 3 µg/ml-18 µg/ml. There were three injections analyzed from each concentration under the same condition. Linear regression analysis was used to evaluate the linearity of the calibration curve through the least square linear regression method.

### Sensitivity

The Limit of Detection (LOD)/Quantitation (LOQ) of quercetin solution was determined by analyzing different solutions and measuring the signal-to-noise ratio. LOD is the concentration that gives a balance of approximately 3:1, while LOQ provides a ratio of 10:1 %RSD (n=3) of less than 10%.

### Accuracy

Recovery studies determined the accuracy of the assay method at three levels of concentration of quercetin load to sample, which are 80%, 100%, and 120% at 160 ppm, 200 ppm, and 240 ppm µg/ml. The percentage recovery of added quercetin and RSD was calculated for each replicated sample.

### Precision

Several measurements of standard stock solution in the morning and evening determined the system and method precision of the proposed methods. Meanwhile, the sample solution was measured three days after, and six assay determinations of standard established method precision.

### Robustness

The method's robustness was verified by applying minor and deliberated changes. The robustness test in this study are Colum temperature (±4 °C); Flow rate (±0.25 ml/min); Waveleght (±5 nm), and Composition of mobile phase (Buffer phosphate: Acetonitrile±5%). In this study, every parameter has chages to evaluate the effect of the method, and the data was estimated by calculating % RSD.

### Stability of analytical solution

Analytical solutions strength was studied by analyzing the standard Preparation at 0h, two days in a refrigerator, at room temperature 30 °C, and 35 °C for 24 hour. Furthermore, five injections from each solution were analyzed to calculate the peak and the RSD average.

**Quantitative analysis of quercetin in sample (Application of the validated analytical methode)**

Ethyl acetate fraction of *Melastoma malabathricum,* as much as 0.1 grams, was added to prepare 100 grams of nano cream. The lipid and water phases are mixed at a temperature of 70 °C and then homogenized at a speed of 750 rpm for 10 min. The nano cream preparation was dissolved in acetonitrile and then homogenized. The sample was injected into the HPLC nine times, and the levels of quercetin obtained were calculated.

**Antioxidant activities with FRAP method**

Antioxidant activity testing with the Ferric Reduction Antioxidant Power (FRAP) method was conducted with minor modifications according to (Apridamayanti *et al.*, 2022) study [7and8]. Meanwhile, 30 µl of each sample at different concentrations of 1 µg/ml to 650 µg/ml were added to 30 µl FeCl3 solution (3 mmol in 5 mmol citric acid) and 240 µl TPTZ reagent (1 mmol in 0.05 M HCL) in a 96-well microplate. Incubation for 20 min at room temperature and absorbance was measured at 615 nm and read with Elisa Reader.

**Identification of *in vitro* sun-protecting factors (SPF)**

The sample was dissolved in ethanol at a concentration of 2000 µg/ml, 4000 µg/ml, 8000 µg/ml, and 10.000 µg/ml and scanned in the range of 290 to 320 nm. Screening sun protection activity was measured by the determination of SPF *in vitro* based on the equation proposed by Mansur [17].

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Note: CF, EE, I, and Abs are the correction factor, Eritema effect in the spectrum (nm), light intensity (nm), and absorbance sample. The absorbances sample was measured three times and was used for SPF calculation.

**Statistical analysis**

The data analysis is presented as means±standard deviation and % RSD. Statistical analysis of photoprotector activity on nano cream samples using IBM SPSS with one sample t-test, where a statistical significance of $p<0.05$ was considered significant.

**RESULTS AND DISCUSSION**

**Result**

The stationary phase in the reverse chromatography column used non-polar silica C-18, namely VP-ODS shim-pack C-18, 250x4.6 (mm). The method is optimized by comparing flow rates 0.25, 0.5, 0.75, and 1 ml/min with a mobile phase of phosphate buffer pH 5.51 and acetonitrile at 45:55 and 60:40 v/v. HPLC detection was carried out at a wavelength, column temperature, and injection volume of 370 nm, 30 °C, and 30 µl. The optimization results are presented in table 1. The result was obtained using phosphate buffer pH 5.51 and acetonitrile with a ratio of 60:40 v/v and a flow rate of 0.5 ml/min, as presented in fig. 1.

**Table 1: Optimation method for validated quercetin as a biomarker**

| Flowrate (ml/min) | Mobile Phase | Wavelength | Observation (Tailing factor) | Result |
|---|---|---|---|---|
| 0.5 | Buffer: Acetonitrile, 45:55 | 370 nm | Tailing factor 1.35 | Method rejected |
| 0.25 | Buffer: Acetonitrile, 40:60 | 370 nm | Tailing factor 1.25 (time-consuming) | Method rejected |
| 0.5 | Buffer: Acetonitrile, 40:60 | 370 nm | Tailing factor 1.045 (Good resolution) | Methode accepted |
| 0.75 | Buffer: Acetonitrile, 40:60 | 370 nm | Broken chromatogram (Poor resolution) | Method rejected |
| 1 | Buffer: Acetonitrile, 40:60 | 370 nm | Broken chromatogram (Poor resolution) | Methode rejected |

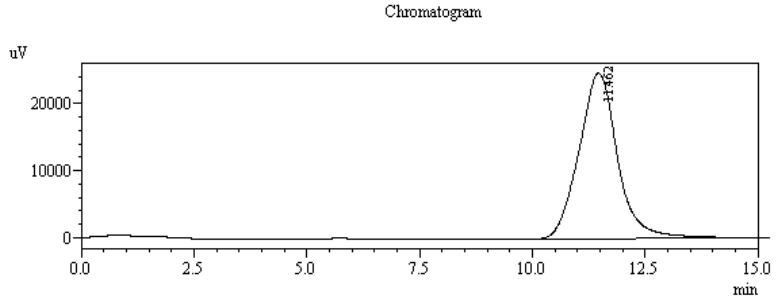


**Fig. 1: Chromatogram of a standard solution of quercetin**

**Method validation**

**Linearity and range**

Linearity is an analysis method that states the ability of the technique to obtain results with a linear comparison between the analyte concentration and the response in a specific range. This study contains six standard solution concentrations of the target chemicals. Each concentration was analyzed three times, and a linearity test was performed on the peak area with different concentration levels. The results of linearity measurements, correlation coefficients, slope, intercept values, and ranges can be seen in table 2 and fig. 2.

**Table 2: Linear regression data for calibration curve of quercetin (N=6)**

| No | Parameters | Quercetin standard |
|---|---|---|
| 1 | Linearity range (µg/ml) | 3-18 |
| 2 | Regression equation | y = 170220x-85497 |
| 3 | Correlation ($r^2$) coefficient | 0.9998 |
| 4 | Slope | 169480.75±0.732 |
| 5 | Intercept | 80829.5±10.475 |
| 6 | LOD (µg/ml) | 0.236 |
| 7 | LOQ (µg/ml) | 0.786 |

Number of experiments, n=3

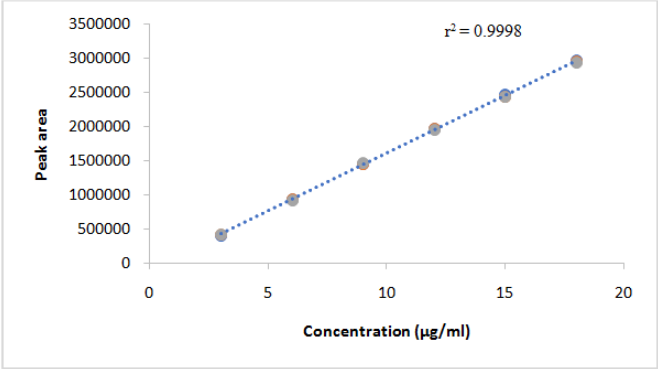


**Fig. 2: Calibration curve of quercetin standard solution**

### Sensitivity

LOD and LOQ are minor concentrations of some analytes that can be detected and calculated quantitatively. LOD and LOQ show that the proposed method provides sensitivity results and can be quantified simultaneously for the analyzed compounds, as seen in table 2.

### Precision

In this study, the precision value of the standard solution was measured using the repeatability method on the same and different days. Measurement of the precision value of the sample solution was carried out by six times analysis. The measurement results determine the RSD value on the area and retention time. The results show that the system and method used are precise, as seen in table 3, with an acceptable RSD value. According to Naseef *et al.*, 2018 the acceptability value of precision is not more than 2% [16].

### Accuracy

Accuracy is an analytical procedure in determining the closeness value obtained from the system with the actual value. According to Naseef *et al.*, 2018, the accuracy values at three concentration levels at the 100% unit level range from 98%-102%. Meanwhile, Borman and Nelder., 2017 stated that analytes with a concentration level of 10 ppm are in the field of 80%-110%, and the RSD value is not more than 2%, as seen in table 4 [18].

**Table 3: System presicion of standard solution quercetin**

| Replicated number | Inter-day | | | | Intra-day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Morning | | Evening | | Peak area | | | Retention time (RT) | | |
| | Peak area | RT | Peak area | RT | Day-1 | Day-2 | Day-3 | Day-1 | Day-2 | Day-3 |
| 1 | 1436189 | 11.438 | 1490858.9 | 11.483 | 1436189 | 1490968.6 | 1465284.2 | 11.438 | 11.438 | 11.471 |
| 2 | 1480770 | 11.458 | 1481108.8 | 11.497 | 1480770 | 1458055.5 | 1471203.1 | 11.458 | 11.466 | 11.468 |
| 3 | 1471292,5 | 11.448 | 1461822.2 | 11.505 | 1471292.5 | 1452042 | 1474555.5 | 11.448 | 11.453 | 11.475 |
| 4 | 1475109.1 | 11.453 | 1513602.2 | 11.508 | 1475109.1 | 1457893.4 | 1492712.6 | 11.453 | 11.44 | 11.479 |
| 5 | 1449412.5 | 11.452 | 1481876.4 | 11.493 | 1449412.5 | 1460550.3 | 1486107.3 | 11.452 | 11.42 | 11.463 |
| 6 | 1475868.8 | 11.464 | 1507670.7 | 11.496 | 1475868.8 | 1477609.1 | 1483726 | 11.464 | 11.402 | 11.458 |
| 7 | 1461389.4 | 11.466 | 1465931.3 | 11.576 | 1461389.4 | 1469758.7 | 1484239.4 | 11.466 | 11.415 | 11.478 |
| 8 | 1486438.4 | 11.559 | 1458673.1 | 11.68 | 1486438.4 | 1488282 | 1497914.3 | 11.559 | 11.413 | 11.508 |
| 9 | 1472302.1 | 11.784 | 1458673.1 | 11.68 | 1472302.1 | 1482212.2 | 1451629.4 | 11.784 | 11.431 | 11.485 |
| 10 | 1501605.6 | 11.712 | 1498272.8 | 11.682 | 1501605.6 | 1489770.4 | 1500974 | 11.712 | 11.426 | 11.454 |
| Average | 1471037.74 | 11.5234 | 1481848.95 | 11.56± | 1471037.74 | 1472714.22 | 1480834.58 | 11.5234 | 11.4304 | 11.4739 |
| | ±18508.39 | ±0.12 | ±20426.52 | 0.09 | ±18508.39 | ±14904.26 | ±15322.83 | ±0.12 | ±0.02 | ±0.02 |
| %RSD | 1.258 | 1.078 | 1.378 | 0.753 | 1.258 | 1.012 | 1.035 | 1.078 | 0.170 | 0.134 |

**Table 4: Recovery data of the proposed HPLC method**

| % Spiked label | Replicated number | Peak area | % recovery | %RSD |
|---|---|---|---|---|
| 80 | 1 | 13510202.8 | 99.839 | 0.009 |
| | 2 | 14416947 | 106.497 | |
| | 3 | 14818493.8 | 109.446 | |
| 100 | 1 | 16971528.4 | 100.205 | 0.002 |
| | 2 | 17050639.4 | 100.670 | |
| | 3 | 17294019.6 | 102.100 | |
| 120 | 1 | 19367229.9 | 95.233 | 0.001 |
| | 2 | 19405970.2 | 95.422 | |
| | 3 | 19475385.8 | 95.762 | |

Number of experiments n = 3

### Robustness

The robustness test shows small changes in methods such as mobile phase, temperature, flow rate, and intense wavelength within acceptable limits, as seen in table 5. This study observed the value of the %RSD area and retention time, where the number of tests was analyzed five times. According to Naseef *et al.*, 2018, the accepted limit for the robustness test is the % RSD value of less than 2% [16].

**Table 5: Robustness data of the proposed HPLC method**

| Parameter | | Peak area (% RSD) | Retention time (%RSD) |
|---|---|---|---|
| Column temperature | 26 °C | 0.776 | 0.045 |
| | 30 °C (Normal) | 1.659 | 0.067 |
| | 34 °C | 0.789 | 0.059 |
| Wavelength | 365 nm | 0.338 | 0.086 |
| | 370 nm (Normal) | 0.610 | 0.069 |
| | 375 nm | 1.481 | 1.642 |
| Mobile phase compotition | +5% acetonitrile | 0.437 | 0.152 |
| | Normal | 0.301 | 0.165 |
| | -5% acetonitrile | 0.935 | 0.107 |
| Flow rate | 0.25 ml/min | 0.186 | 0.141 |
| | 0.35 ml/min | 0.744 | 0.318 |
| | 0.5 ml/min (Normal) | 0.981 | 0.167 |

*Number of experiments n = 3

**Standard solution stability**

The stability of the solution was assessed by determining the standard at 24 h, 48 h, and 96 h at 30 °C. The solution was stored in the refrigerator at a temperature of-19 °C and held for 24 h at 35 °C. Analysis was carried out for five injections in each test condition, and the RSD parameters used were the area and retention time. The value with the acceptable limit was not more than 2.0% [16], as seen in table 6.

**Quantification of quercetin content in nano cream samples**

Analysis of the quercetin content was performed in the nano cream preparation using a method that has been previously validated. The instrument used was RP-HPLC, and quercetin was reported in nano cream preparation. Retention Time (RT) of quercetin is known at 11.41 min and amouth of quercetin in nanocream foundation is $0.021728 \pm 5.83 \times 10^{-4}$ mg/g sampel.

***In vitro* assay**

**Antioxidant activity with the FRAP method**

The activity test for nano cream was carried out by dissolving it in ethanol solvent with a concentration range of 800 g/ml–2800 g/ml. Antioxidant testing method using FRAP reagent: The sample was reacted with FeCl3 in an acid atmosphere, and the TPTZ reagent was incubated for 15-30 min. The absorbance was read using an ELISA Reader at a wavelength of 615 nm. The results can be seen in table 7 with an R2 value of 0.9573, and the IC50 in the nano cream preparation was 1444.157±15.538 g/ml.

**Table 6: Standard solution stability data of the proposed HPLC method**

| Parameter | Peak area (%RSD) | Retention time (%RSD) |
|---|---|---|
| After 24 h at 30 °C | 0.750 | 0.110 |
| After 48 h at 30 °C | 1.034 | 0.087 |
| After 96 h at 30 °C | 0.264 | 0.142 |
| After 24 h at 35 °C | 0.897 | 0.113 |
| After 24 h at-19 °C | 1.659 | 0.067 |

*Number of experiments n = 3

**Table 7: Measurement of the antioxidant activity of nano cream samples**

| No | Concentration (µg/ml) | % Inhibition | |
|---|---|---|---|
| 1 | 800 | 34.405 | y = 0.0233x+15.893 |
| 2 | 1200 | 45.083 | $R^2$ = 0.9573 |
| 3 | 1600 | 52.843 | $IC_{50}$ = 1444.157±15.538 µg/ml |
| 4 | 2000 | 57.490 | |
| 5 | 2400 | 77.831 | |
| 6 | 2800 | 78.968 | |

Number of experiments, n=3

**Photoprotector activity**

The activity of measuring photoprotector activity uses the SPF value parameter. The test absorbance values in the 2000 g/ml concentration range–10000 g/ml at 290-320 nm wavelength. Furthermore, the measurements using the Mansur formula, as shown in table 8. In the measurement results, the SPF value was good at 8000 g/ml and 10000 g/ml concentrations.

**Table 8: Measurement of photoprotector activity on nano cream samples**

| No | Concentration (µg/ml) | SPF value |
|---|---|---|
| 1 | 2000 | $2.163 \pm 0.332^a$ |
| 2 | 4000 | $6.419 \pm 2.000^b$ |
| 3 | 8000 | $11.693 \pm 1.251^c$ |
| 4 | 10000 | $11.370 \pm 1.083^c$ |

*Values are expressed as mean±SD, Number of experiments, n=3, *a,b,c have a significant difference value P<0.05

**DISCUSSION**

Nanotechnology is a development innovation that includes designing, characterization, producing, and modeling structures with shapes and sizes in the nanometer scale of 1 nm to 100 nm [19]. Some forms of nanotechnology commonly used in the cosmetic field are nanodots, liposomes, dendrimers, and nanoemulsions. Nanoparticle-based cosmetics are developed to treat wrinkled skin, dehydration, premature aging, and hyperpigmentation [20]. Nanotechnology has an excellent opportunity to be designed for industries engaged in cosmetics and is considered the latest and most promising technology.

UV rays are divided into three categories based on their wavelength: UV A, B, and C at 320-400 nm, 280-320 nm, and 200-280 nm. UV C is entirely absorbed by oxygen and ozone in the atmosphere. In contrast, the skin can absorb UVA, and B. UV rays can cause skin problems such as sunburn, premature aging, photocarcinogenesis, inducing skin cancer, and immunosuppressants [1]. The exposure results in MMP-1 expression, collagen degradation, and inflammation. The involvement of ERK, JNK, Akt, and STAT3 signals can induce an inflammatory process that can activate the expression of MMP-1 and COX-1 [21].

Utilization of the use of quercetin in cosmetics has a great opportunity. With the help of nanotechnology, nano cream preparations were made through ethyl acetate fraction of *Melastoma malabathricum* leaves. Furthermore, *in vitro* studies through antioxidant activity and SPF values can provide an initial picture of the potential for making nano-cream preparations.

This study validated a single laboratory through linearity, accuracy, repeatability, sensitivity, roughness, and stability test parameters. The optimization method in this study where the standard of quercetin solution dissolved with Acetonitrile (ACN), then injected into the HPLC system using acetonitrile and phosphate buffer pH 5.1 at a wavelength of 370 nm with retention time (RT) is 11.483 min. It obtained an exemplary chromatogram with a tailing factor value of 1.045, presented in table 1, according to Synder *et al.*, 2010 and Kurshed *et al.*, 2021 [22, 23]. Literature study of quercetin in the formulation Chaudari *et al.*, 2020 study chromatographic condition for quercetin in nanostructure lipid formula is Glacial Acetic Acid (GAA) 2% pH 2.6: Acetonitril (ACN) with colom chromatographic is ODS C-18 150 mm x 4,6 mm, flow rate 1 ml/min, wavelength 346 nm value of RT and TF are 2.837 min and 1.54 [24]; Patel *et al.*, 2020 study in bulk drug microsphere formulation, the chromatographic condition is methanol: ACN (50:50) with colom chromatographic is ODS C-18 250 mm x 4,6 mm, flow rate 1 ml/min, wavelength 256 nm, the value of RT and RF is 4.186 min and 2.8; Khursheed *et al.*, 2021 study quercetin in the market and SNEDDS formulation, the chromatographic condition is GAA 2% and ACN with gradient elution 40-80% during 15 min, with colom chromatographic is ODS C-18 250 mm x 4,6 mm, the flow rate is 1 ml/min value of RT and TF are 6.064 and 0.918 [23, 30]. The study results show that the validation system developed by the researchers is no different from previous studies. The difference in retention time (RT) is caused by differences in flow rates in the chromatography system, where in this study flow rate is 0.5 min.

The optimization in table 1 uses the mobile phase carried out and then continues in the linearity test. In this study, the test was evaluated according to the recommendations by ICH using six types of concentrations to show linearity through statistical evaluation. The results showed that the R2 value was 0.9998 with a y = 170220x–85497, presented in table 2. According to Naseef *et al.*, 2018, a good correlation value is close to 1 [16]. The correlation value shows a linear relationship between the analyte concentration and the area under the peak. LOD is obtained from a linearity test that can be clearly distinguished from the baseline. The resulting response is more significant than three times the baseline noise response [25].

Meanwhile, LOQ is the concentration that produces a detector signal ten times greater than the baseline noise response. The study results in table 2 obtained a LOD value of 0.236 µg/ml and a LOQ of 0.786 µg/ml. Study Vaz *et al.*, 2020 standard solution quercetin development in LOD and LOQ nanoemulsion values are 0.14 µg/ml and 0.48 µg/ml [29]. In the quercetin study in SNEDDS formulation by Ahmed *et al.*, 2021 LOD and LOQ are 7.65 ng/ml and 23.19 ng/ml [26].

The precision determines the closeness value in a series of measurements from several samples under specified conditions, and the result is the Relative Standard Deviation (RSD). It is performed according to ICH guidelines by repeating on the same and different days. According to the ICH guidelines, repeatability is conducted at least six times at a concentration of 100% [27]. In this study, ten analyses were performed at a concentration of 100% with a parameter repeatability value (RSD) of less than<2%, as seen in table 3. A good repeatability value that indicates the equipment's ability to produce reliable data will also be demonstrated during the analysis process.

The accuracy was measured using the spiking technique, where several analytes were added to the standard solution at 80%, 100%, and 120%. Furthermore, the recovery value was 95.233%-109.446%, with RSD<2%. The percentage under the specified limits indicates that the proposed method is well-validated and suitable for quantitatively detecting the quercetin content in the nano cream preparation, as seen in table 4.

Robustness is an analytical method for evaluating the effect of minor modifications in HPLC conditions on the system suitability parameters of the developed method [16]. According to Caesar and Pianetti, 2009, the impact of minor changes consists of mobile phase composition, pH and ionic strength, temperature, and different lots or suppliers of columns. The validation process cannot be separated from the actual condition's development of the procedure [28]. In this experiment, this study analyzed variations in column temperature, wavelength, the composition of the mobile phase, and flow velocity, it was done with repetitions, and the RSD value was<2%, as shown in table 5. From the results obtained, the robustness of the analytical method developed has properties that are not easily influenced by changes in operational parameters known to the process results [11].

The solution testing method shows more complex stability because it can form several unknown degradations under different stress conditions [11]. The strength of the solution was tested against the standard at different temperatures and time conditions, and the results showed an RSD value <2%, as seen in table 6. The study, equal to Vaz *et al.*, 2020 result from this study indicated the stability of quercetin standard in different temperatures showed an RSD value is 0.4-1.87 [29].

The application of the proposed method is evaluated as a quantitative analysis of the validation results in the developed process that is accurate, precise, reliable, and used in marker analysis. A study of quercetin compound in crude extract and ethyl acetate fraction in *Melasthoma malabatricum* was conducted by Apridamayanti *et al.*, 2022[a]. The composition of quercetin in crude extract and ethyl acetate fraction is 0.40±2.03 and 0.93±0.06 mg in each gram sample [8]. The survey results obtained were 2.1728x 10-2±5.83x $10^{-4}$ mg in each gram of nano cream preparation. From Petel *et al.* 2020 study is known that the determination of quercetin shows that the HPLC method is more accurate and precise. HPLC methods which are known to be specific, fast, and accurate, can be used for routine control analysis of pharmaceutical dosage forms [30].

Examining the content of markers in practices derived from natural ingredients aims to meet the therapeutic aspects of the nano cream preparations. Quercetin is a flavonoid with highly effective antioxidant and anti-inflammatory activities suitable for skin supplements. This compound exhibits exciting actions in cellular and animal-based models, ranging from protecting cells from UV irradiation to supporting skin regeneration in wound healing. However, quercetin has limited skin penetration ability due to its poor solubility, and various formulation approaches are taken to increase its dermal function [31]. The development of the compound in the cosmetic field has been carried out by Hatahet *et al.*, 2016, and the nano-dose form shows higher deposition in the upper skin layer of the epidermis [31]. The particle size is minimal in the form of a nano dose. Still, the development of the delivery system seems to be

the primary determinant of the extent to which the depth can penetrate the skin layer. Shin *et al.*, 2019 showed that the compound could suppress ERK, JNK, Akt, and STAT3 signals involved in the expression of COX-2 and MMP-1. The presentation of COX-2 and MMP-1 is due to stimulation from UV light and can inhibit the expression of genes involved in skin aging [21].

The previously known therapeutic effect determined the impact of nano cream preparations in which the ethyl acetate fraction of *Melastoma malabathricum* leaves was found *in vitro*. Antioxidant activity was examined using the FRAP method to reduce $Fe^{3+}$to $Fe^{2+}$ through $Fe^{3+}$-triptidyltriazine ($Fe^{3+}$-TPTZ). This interaction forms an indicative color due to the complex formed between quercetin and $Fe^{2+}$-TPTZ compounds at the maximum absorbance wavelength (Apridamayanti *et al.*, 2022), as seen in table 7 [7]. Furthermore, the $IC_{50}$ value of nano cream preparations is 1444.157±15.538 g/ml, and the prepared nano cream has antioxidant activity. The *in vitro* test related to the effect was continued with the SPF test, which correlated with quercetin's photoprotective properties against UV rays. Table 8 shows that the SPF test was performed using four different concentrations of nano cream preparations. The 8000 ppm and 10,000 ppm concentrations have the best SPF values of 11.693±1.251 and 11.370±1.083. Following the study of Hasim *et al.*, 2019, the SPF value of bemotrizinol nanoemulsion preparation is 16.08±0.39. It's known that a formula with nanoemulsion preparation provides good protection against UV rays with good SPF value while maintaining its photostability. The SPF value obtained is the maximum protection against UV rays [32].

## CONCLUSION

The validation results of the developed process show the RP-HPLC method with phosphate buffer pH 5.51 mobile phase and acetonitrile at a ratio of 60:40 v/v with a flow rate of 0.5 ml/min. The detection was carried out at a wavelength, temperature column, and injection volume of 370 nm, 30 °C, and 30 µl. The development method of quercetin is known to be simple, accurate, precise, sensitive, and robust. Quantitative analysis of quercetin biomarkers in nano cream preparations containing the ethyl acetate fraction of *Melastoma malabathricum* leaves using the RP-HPLC method yielded ($2.1728x\ 10^{-2}$±$5.83x\ 10^{-4}$ mg/g). Meanwhile, the prepared nano cream has a therapeutic effect as indicated by the results of antioxidant activity with an $IC_{50}$ and SPF values of 1444.157±15.538 g/ml and 11.693±1.251.

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## AUTHORS CONTRIBUTIONS

All authors have contributed equally. AP prepare the manuscript and data analysis; PL prepare the nanocream foundation and SR as technical support in the laboratory.

## CONFLICTS OF INTERESTS

The author declares that there have been no conflicts of interest.

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