RESEARCH PAPER

Extraction, Physicochemical Profiling, and Rp-Hplc Method Development for Biterin Oil

[1]Sakshi Sharma, [1]Rakesh Kumar, [1]Dileep Singh Baghel, [1]Ankita Banerjee, [1]Prince Kumar and [1*]Rajesh Kumar

[1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India*
[1]sakshis3011@gmail.com, [1]sharmarakesh846@gmail.com, [1]baghel_12@rediffmail.com,
[1]ankitabanerjee730@gmail.com, [1]priince96310@gmail.com and [1]rajksach09@gmail.com
***Corresponding author:** Dr. Rajesh Kumar/rajksach09@gmail.com*

Received: 14th June, 2026; Revised: 20th July 2026; Accepted: 20th August, 2026; Available Online: 05th September 2026

ABSTRACT

Objective: The objective of the present study was to extract biterin oil and evaluate its physicochemical characteristics. The RP-HPLC was developed and validated for qualitative and quantitative estimation of biterin oil.

Method: The Soxhlet extraction method was used to extract biterin oil from seeds of *Momordica charantia*. The acid value, saponification value, iodine value, and refractive index were estimated for the extracted oil. The FTIR (also compared with marketed oil) and GCMS analysis of the oil were conducted. For the RP-HPLC method development and validation, the concentration of acetonitrile and flow rate were the considered parameters. The responses were determined by observing peak area and retention time.

Results: The Soxhlet extraction method yielded 55% oil. The higher saponification value and iodine value indicated the presence of a higher amount of fatty acids. The FTIR spectrum analysis showed various groups indicating the presence of saturated, monounsaturated, and polyunsaturated fatty acids. The biterin oil trans-esterified for GCMS analysis exhibited the spectrum of linoleic acid, known for its potency in the wound healing process. The RP-HPLC method was validated, and the findings for linearity, accuracy, precision, LOQ, LOD, and robustness were within the limits.

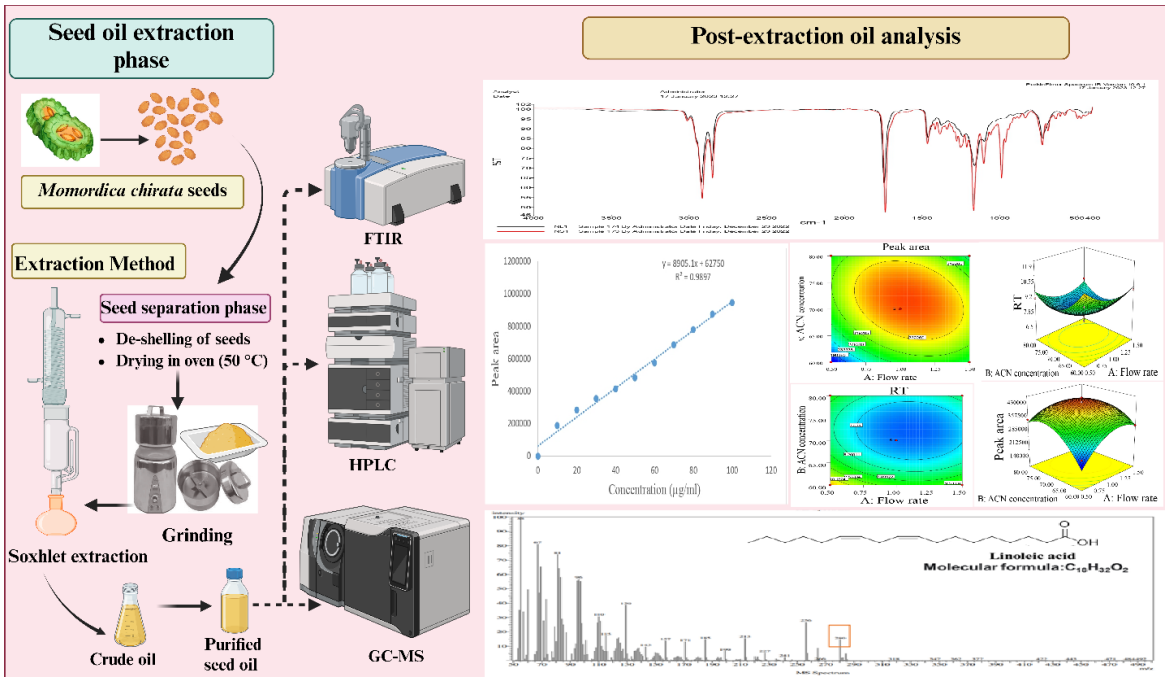
Conclusion: The Soxhlet extraction method was found to be highly efficient with a significant yield. The RP-HPLC method developed for extracted biterin oil was suitable, with a retention time of less than 10 minutes. To optimise chromatographic conditions, we applied a central composite design, and the developed method was validated for estimating biterin oil in further studies.

Keywords: Biterin oil; *Momordica charantia*; Soxhlet extraction; Physicochemical characterization; RP-HPLC method development; Method validation.

How to cite this article: Sharma S, Kumar R, Baghel DS, Banerjee A, Kumar P, Kumar R., Extraction, Physicochemical Profiling, and Rp-Hplc Method Development for Biterin Oil. Int J Drug Deliv Technol. 2026; 16(8): 285-294. DOI: 10.25258/ijddt.16.8.29
Source of support: Nil.
Conflict of interest: None

*****Author for Correspondence: rajksach09@gmail.com*

GRAPHICAL ABSTRACT



1. INTRODUCTION

Preformulation studies support the rational development of formulations by identifying the physicochemical and biopharmaceutical characteristics of the drug moiety. The physicochemical properties strongly influence the efficiency of the dosage form [1]. These studies involve the evaluation of parameters like pKa, solubility, partition coefficient, flow behaviour, polymorphic forms, and chemical and physical stability characteristics [2]. The preformulation studies of extracted constituents derived from plant or microorganism derivatives suffer batch-to-batch variability due to differences in source, extraction method, and purification processes. The selection of appropriate carriers, encapsulation systems, and chemical modifications can be identified by evaluating preformulation parameters to enhance bioavailability and reproducibility of formulations [3]. It is useful to develop a safe and efficacious dosage form with a suitable delivery system, as poor properties lead to higher variability, poor clinical/preclinical models, and also enhance the cost of development [4].

Nowadays, it has been observed that research is mainly focusing on the use of herb-derived nutraceuticals. There is great importance of fats and oils in the human diet, which have derived them as a source of industrial raw material. The market demand for edible oils that are extracted from plants is rising. There are many wild plants from which seed oil is extracted and is in high demand. *Momordica charantia*, commonly referred to as karela, bitter melon, or bitter gourd, belongs to the family Cucurbitaceae. This plant is grown in humid and subtropical regions of the world. This plant has great applications in therapy and the food industry [5]. The biterin oil consists of carpic acid, lauric acid, palmitic acid, stearic acid, Oleic acid, arachidic acid, and linoleic acid. It has been reported that at concentrations of 2 mg/mL and 125 to 500 µg/mL, the *Momordica charantia* seed oil (biterin oil) exhibits α-glucosidase inhibition and antimicrobial characteristics, respectively [6]. The oil is thick, reddish-orange in colour, and bitter in taste. The extracted oil is reported to have high purity, and it is water-immiscible but miscible with organic solvents like benzene, petroleum ether, and acetone. The constituents present in the oil are reported to have antimicrobial and anti-inflammatory activities [7].

2. MATERIAL AND METHODS

The *Momordica Charantia* seeds were purchased from a local vendor in Phagwara, and the Soxhlet assembly was used for extraction as shown in Figure 1. The authentication of seeds was carried out at Punjab Agricultural University, Ludhiana, Punjab, India. Initially, seeds were subjected to 3-4 rounds of rinsing with tap water. Subsequently, the seeds were dried in a hot air oven at a temperature of 50 °C, followed by pulverization using a grinder. Then, the seeds that were activated by a power source were relocated into a cellulose thimble measuring 30 mm in length and 20 mm in width. In the extraction chamber of the Soxhlet apparatus with a condenser, the thimble was introduced and placed over a 500 mL distillation flask containing 250 mL of n-hexane. The oil extracted from the seed was subjected to extraction and refluxing with n-hexane for varying durations of 1, 2, 4, and 6 hours, with a frequency of 10-12 cycles per hour. The hexane was eliminated utilizing a rotary evaporator, and the extracted oil's yield was computed [5].

2.1. Characterization of biterin oil

2.1.1. Acid value

A 250 mL beaker was used to heat a mixture of 50 mL of ethanol and 5 g of oil sample until the boiling point was

reached. Then 0.1 N KOH was used for titration against the final product with addition of phenolphthalein as an indicator. The mixture was stirred continuously till pink hue was observed.

2.1.2. Saponification value

In the conical flask, the biterin oil (5 g) and 0.1 N ethanolic KOH (25 mL) were added. The mixture was set to reflux for 1 hour. After that, it was titrated against 0.5 M HCl using phenolphthalein as an indicator till it became colorless. Then, the saponification value was calculated.

2.1.3. Iodine value

The oil (0.2 g) and chloroform (20 mL) were added to a 500 mL conical flask. For 30 minutes, the mixture was kept aside. Then, Potassium iodide (20 µL) was added and homogenized thoroughly. The mixture was titrated with 0.1 N $Na_2S_2O_3$ using starch as an indicator to extract iodine from the chloroform layer by vigorous shaking [8].

2.1.4. Refractive Index

The refractometer was calibrated using distilled water. It was cleaned using acetone and followed by desiccation using cotton material. A small quantity of oil was placed in the space between the prisms. The telescope was rotated for the alignment of the borderline. The measurement of refractive index was conducted under standard room temperature conditions [9].

2.1.5. FTIR (Fourier Transform Infrared Spectroscopy) analysis

To identify the structure and purity of the drug, Biterin oil was analyzed using FTIR. In this, the sample was placed in the respective KBr disc for analysis. The obtained spectrum was recorded in the wavenumber range between 4000 and 400 $cm^{-1,}$ and its characteristic bands of raw biterin oil were compared with the standard spectrum [10, 11].

2.1.6. Compound Identification by GCMS

The solution containing 0.5 g of seed oil with 1 mL benzene, 1 mL methanol, and 2 mL sodium methoxide were prepared. The aforementioned prepared solution was added to a formulation tube that was stoppered and subsequently immersed in water maintained at 100 ℃ for a duration of approximately 45 minutes. The prepared solution was allowed to attain ambient temperature, and 10 mL of distilled water was introduced to facilitate the partitioning of the soluble compounds. After the separation of soluble compounds, n-hexane through a series of three extractions was used for the extraction of methyl esters, with each extraction utilizing 5 mL n-hexane. Rotary evaporator was used to remove hexane from the extract. The trans-esterified biterin oil was submitted to the laboratory for assessment via GCMS.

The biterin oil was subjected to trans-esterification. The GCMS instrument specifications have been adjusted under the following conditions: the analytical tool utilized for analysis is a Shimadzu gas chromatography mass spectrometer. The column was 30×0.32 mm ID and 0.25 µm in film thickness. The carrier gas utilized in the experiment was helium, with a 1.47 mL/min flow rate and 1:0 split ratio. The analysis was conducted utilizing a 40 ºC temperature for a duration of 2 minutes, with a subsequent increase in temperature at 4 ºC/min until reaching 300 ºC, followed by a 2-minute hold period. 1:10 biterin oil sample diluted in hexane was subjected to injection, with an aliquot of 1µl. The acquisition of mass spectra was conducted through electron impact ionization at a level of 70 electron volts, utilizing a spectral range of m/z 20-480 atomic mass units.

For the segregation of soluble compounds the mixture was cooled to ambient temperature and the 10 mL of water was added. After the separation of soluble compounds, the methyl esters were subjected to a series of three successive extractions using 5 mL n-hexane for each extraction. Subsequently, the hexane within the extract was eliminated via the employment of a rotary evaporator. GCMS utilized helium as the carrier gas, operating at a pressure of 81.8 kPa. The experiment was initiated by establishing an initial temperature of 40 °C within the oven and setting the H_2 and air to 81.8 kilopascals as the initial condition. The capillary column underwent an initial conditioning procedure that spanned a duration of one hour, during which it was maintained at 40 °C. The experimental parameters encompassed the establishment of the detector and injector ports at a temperature of 250 °C, programming the capillary column's temperature to uphold the initial temperature of 40 °C for a duration of 2 minutes, followed by an escalation at a rate of 4°C/min until attaining 300 °C, and maintaining it for 2 minutes to facilitate analysis. After introducing µl of concentrated trans-esterified biterin oil into the injector port, the temperature program was initiated and subsequently maintained at 250 °C.

2.2. HPLC analytical method development

High-Performance Liquid Chromatography (HPLC) is an analytical procedure utilized to separate, identify, and quantify various components in a mixture. This process is a leading-edge version of liquid chromatography that uses high-pressure conditions to separate components. The method has two phases: a stationary phase as a packed column and a mobile phase that flows through it under high-pressure conditions [12]. In this method, various analyte interactions take place, which results in varying retention times for each component in a mixture.

2.2.1. Apparatus

For the quantification and measurement of components, HPLC analytical method development is significant. The RP-HPLC method development and validation utilized a reverse-phase C_{18} column [13].

2.2.2. Biterin oil solubility measurement in different solvents

The biterin oil was added to each solvent (10 mL) in excess amount and sonicated for 1 h at 35±2°C. The mixture was allowed to cool till it reached room temperature. After 24 hours, for 10 minutes the mixture was centrifuged at 8000 rpm, and the supernatant was

dissolved in an appropriate solvent and quantified. The recovery method was estimated in mg mL^{-1} for each solvent [14].

2.2.3. Preparation of stock and standard solutions

For the preparation of the stock solution, 20 mg of biterin oil was dispersed in 100 mL of solvent (acetonitrile), from which, in the range of 10-100 µg mL^{-1}, different standard solutions were prepared to obtain the standard curve.

2.2.4. Chromatographic conditions

The biterin oil was analysed in a C18 (150.0 × 46.0 mm, 5µm) column with a mobile phase composition of acetonitrile: water (70:30, v/v %) using a flow rate of 1 mL/min in an isocratic mode. The solvent was filtered using a syringe filter (pore size 0.22 µm, diameter 25 mm), and it was observed that the mobile phase was stable for more than 5 days without any physical changes, maintaining all stability parameters. The wavelength was set to 238 nm. The temperature conditions were maintained at 35± 2°C, and the injection volume was 20 µl [15].

2.2.5. Method validation

The developed method needs to be validated through the analytical parameters like accuracy, precision, linearity, specificity, limit of detection (LOD), and limit of quantification (LOQ) in accordance with ICH guidelines.

2.2.6. Linearity

The biterin oil standard solutions in the concentration range of 10-100 µg/mL were introduced into the reverse-phase HPLC system. The response was recorded at 238 nm for each sample in triplicate. To calculate the regression equation, the calibration curve was plotted.

2.2.7. Detection and quantification limits

The LOQ and LOD were determined in accordance with ICH guidelines as mentioned in equation 1 and 2, respectively. The LOD is defined as the least amount of analyte which is analyzed qualitatively, whereas the LOQ is the least amount of analyte that can be analyzed quantitatively with accuracy and precision [16]. The formulae used to calculate these parameters were:

$LOQ = 10 * \sigma/s$…….. eq 1

$LOD = 3.3 * \sigma/s$…….. eq 2

2.2.8. Accuracy

The close relationship between the true value and the tested value in HPLC defines the accuracy of the method. The method implies calculation of recovery values at different concentrations (25, 50, and 75 µg/mL). The method involves spiking the concentration of the active drug sample at three different amounts, namely LQC (low-quality control), MQC (medium quality control), and HQC (high-quality control). The concentration of active sample recovered was compared to the amount added.

2.2.9. Precision

The solutions of different concentration values (25, 50, and 75 µg/mL) were spiked six times to evaluate the precision of the method. This process was evaluated according to three parameters, mainly repeatability, in which six-times-spiked concentration determinants are considered: intraday, interday, and inter-analyst. In this, the variation is observed by performing analysis for consecutive 3 days and at different intervals on the same day. The percentage recovery was observed based on peak area and compared. The %RSD value should not exceed 2% as an indicator of precision.

2.2.10. Robustness

For the investigation of the robustness of the validated method, small variations were made in factors such as wavelength and flow rate. The percentage recovery was assessed to observe the quantitative effect of these variables, and the acceptable limit for the peak response and retention time of an analyte must be within ±2% variation [17].

3. RESULTS AND DISCUSSION

The formula used for the calculating percentage yield of extracted oil:

$$Extracted\ oil = (Extracted\ biterin\ oil\ weight\ /bitter\ gourd\ seed\ powder\ weight) * 100$$

which was found to be 55.8%. This method of extraction was found to be highly significant, with a high yield in less time. The oil extracted from methods like Soxhlet and stirring differs in the composition of fatty acid profiles in comparison to cold press or superheated hexane extraction. The composition of unsaturated fatty acids varies in the cold-press method. The oil extracted was evaluated for various parameters, as given in Table 1.

Table 1. Physicochemical characteristics of extracted Biterin oil

Characteristics	Observations
Saponification value (mgKOH/g)	145.86
Refractive Index (20 °C)	1.44
Refractive Index	111.67
Iodine value (gI2/100 g)	111.26
Acid value (mg KOH/g)	4.312

The saponification value of oil is 145.86, which depicts the presence of a higher amount of fatty acids, and the iodine value indicates the mass of fatty acids present in the oil. The value of refractive index indicated the presence of conjugated hydrocarbons in substantial amount. The presence of significant amount of fatty acids was indicated by higher saponification value and moderate iodine values

which further added to the potential use of oil in various formulations.

FTIR (Fourier Transform Infrared Spectroscopy)

the FTIR analysis of extracted biterin oil showed wave number 1736.38 that corresponds to the C=O stretching vibration, 2849.81 cm^{-1} corresponds to the OH group and 2916.05 cm^{-1} revealed C-H stretching. The presence of polyunsaturated fatty acids was detected with a prominent peak at 986.37 cm^{-1} .The biterin oil sample revealed mainly the absorption bands corresponding to long-chain fatty acids [18]. The FTIR analysis overall showed a consistent mixture of saturated fatty acids and monounsaturated and polyunsaturated fatty acids. The IR spectra of extracted oil from seeds of *Momordica charantia* completely overlapped the spectra of standard biterin oil, as shown in **Figure 2**.

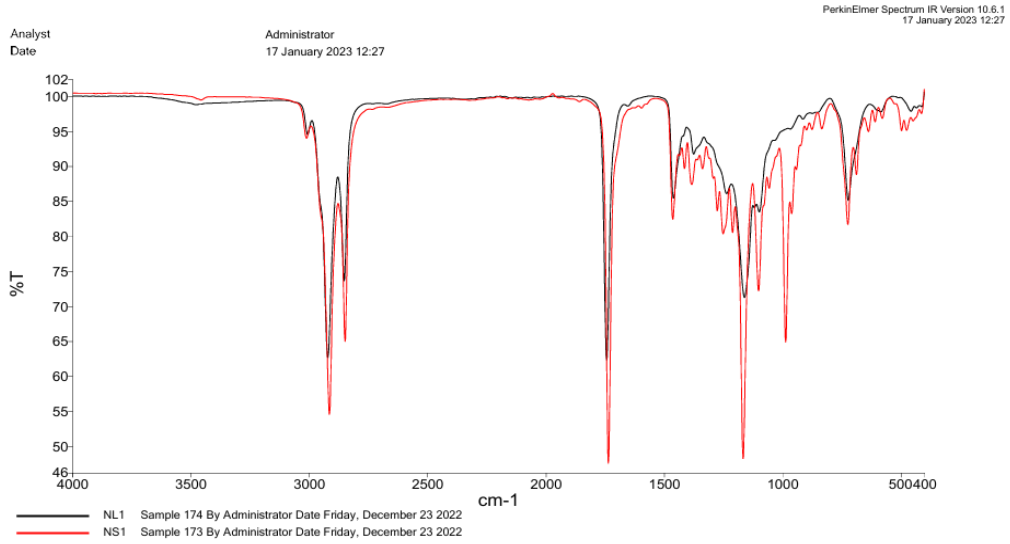


Fig. 1: Overlay IR spectrum of extracted oil sample and reference standard

3.1. GCMS analysis

The process of GC-MS analysis was conducted in triplicate for every sample of the transesterification of biterin oil solution. The mass spectrometry analysis yielded a result of 280 m/z, while the obtained compound exhibited a retention time of 6.18. The sample was found to contain a spectrum of linoleic acid. **Figure 3** depicts the chemical constituents of BGO identified through the use of GC-MS software [19].

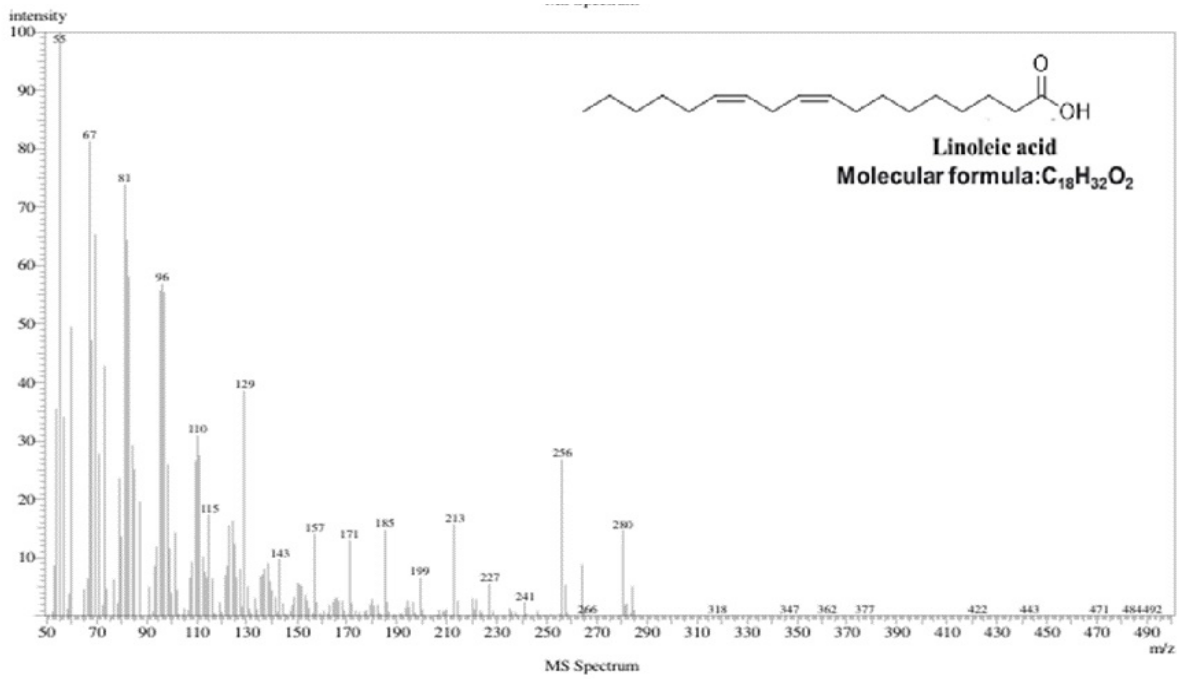


Fig. 2: GCMS analysis spectrum of biterin oil

3.2. Optimization of HPLC experimental parameters

The initial trials in the HPLC method were performed to select the appropriate chromatographic conditions, such as solvent selection, composition of mobile phase, pH, detecting wavelength, column type, and concentration range of standard solutions [20].

3.3. Experimental solubility of Biterin oil

It was important to predict the solubility of biterin oil in various solvents and solvent combinations. The explored solubility data can help to predict the performance of the developed method within the prescribed conditions. The solubility of the oil was found to be maximum in acetonitrile and least in water, as shown in **Figure 4.**

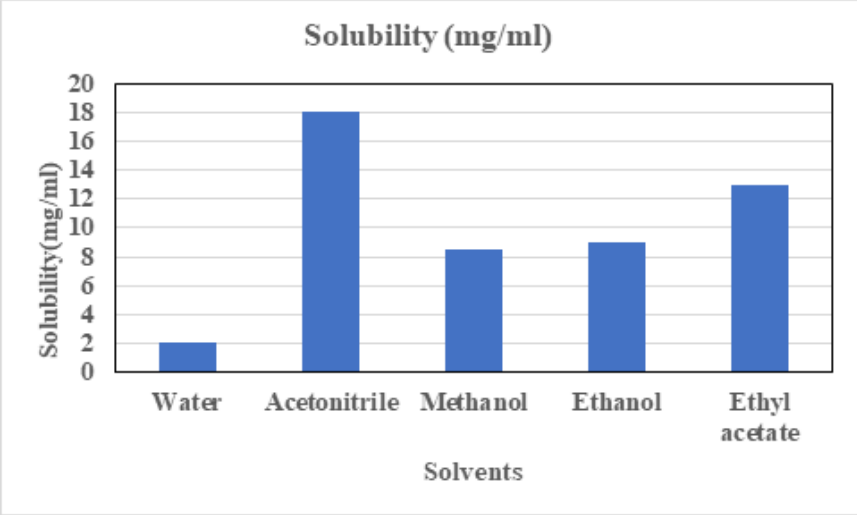


Fig. 3: Experimental solubility of biterin oil in predicted solvents

3.4. Selection of mobile phase

Various solvent systems were used in trials to select a suitable composition for HPLC analysis, as shown in **Table 2**. The various ratios and flow rates of acetonitrile: water, acetonitrile:0.1% orthophosphoric acid, methanol: water, methanol: 0.1% orthophosphoric acid, methanol: phosphate buffer (pH 6.5), and acetonitrile: methanol: water was taken. Finally, acetonitrile: water was selected in the proportion 70:30, which produced a sharp peak with the desired retention time. Methanol gave a longer run time, and broadening of the peak occurred. Acetonitrile gave good peak separation with the sample.

Table 2. HPLC trial batches

S No.	Mobile phase	Ratio (%v/v)	Flow rate (mL/min)	Observations
1	Acetonitrile: water	60:40, 80:20	1	Slightly broadened peak with long retention time
2.	Acetonitrile: water	90:10	1	The peak was broadened
3.	Acetonitrile: 0.1% Orthophosphoric acid	60:40, 80:20, 70:30	1	The peak was broadened
4.	Methanol: Phosphate buffer (pH 6.5)	60:40, 80:20, 70:30, 90:10	1	No peak was observed
5.	Methanol:0.1% Orthophosphoric acid	60:40, 80:20, 70:30, 90:10	1	The peak was negligible
6.	Acetonitrile: Methanol: Water	70:20:10, 60:30:10, 50:40:10	1	A small peak was observed
7.	Acetonitrile: water	70:30	1	Sharp peak with desired retention time

3.5. Selection of detection wavelength

The spectrum of biterin oil was recorded in the wavelength range 200-400 nm. The peaks were recorded at 238 nm and 269 nm. The highest absorbance was observed at 238 nm, so it was selected further for HPLC analysis.

3.6. Central composite design (CCD)

The CCD was used after a preliminary assessment of variables. Thirteen experimental runs were designed by employing two significant factors. **Table 3** shows the matrix of CCD. The aim of utilizing DOE in method development and validation is to have an HPLC method that is robust, accurate, and precise for the quantification of biterin oil. This design represents a quadratic polynomial equation (1) between the factors as:

$$Y = \sigma + \sigma_1 X_1 + \sigma_2 X_2 + \sigma_3 X_1 X_2 + \sigma_4 X_1^2 + \sigma_5 X_2^2 \ldots\ldots$$ (eq. 1.)

Here, the Y signifies dependent variables, $\sigma_1$and σ_2 are the coefficients of X_1 and X_2. The $\sigma 0$ and $\sigma 3$ represent the

intercept and the coefficient of interaction between the factors X_1 and X_2. **Figure 5** shows the significant direct impact of the concentration of ACN on the peak area. The peak area increases with the increase in concentration. Therefore, the DOE suggested the combination of ACN and water to avoid broadening of the peak. The flow rate also exhibited a remarkable impact on peak area and retention time. Therefore, the ACN concentration and flow rate were optimized to obtain a sharp peak with maximum area [20].

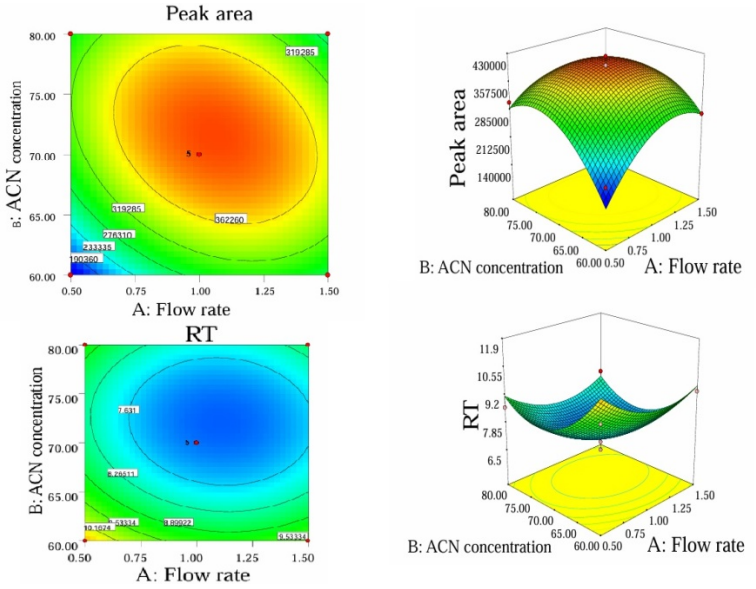


Fig.4: 2D and 3D response surface plots depicting the influence of critical factors on the dependent variables, Peak area and retention time

Table 3. Central Composite design and Statistical analysis of applied factors

Independent variables at three levels			**Dependent variables**		**Goals**		
Design run	A	B	Retention time (min)		Minimum		
1	1	70					
2	1.7	70	Peak area (mAU)		Maximum		
3	0.2	70					
4	0.5	60					
5	1	70					
6	1.5	80					
7	1	84					
8	1	55					
9	1.5	60					
10	1	70					
11	1	70					
12	0.5	80					
13	1	70					
Factors	**Minimum**	**Intermediate**	**Maximum**	**Model**	**P value**	**F value**	**R^2**
A(X1):ACN concentration(%v/v)	55	70	85	Quadratic	0.0012	15.34	0.91
B(X2): Flow rate	0.2	1	1.7	Quadratic	0.0075	8.27	0.94

Table 4. Selected Chromatographic Conditions

Parameters	**Optimized conditions**
Stationary phase	C18 (150.0 × 46.0 mm, 5µm) column

Composition of Mobile Phase	Acetonitrile: water
Mobile phase ratio	70:30
Injection volume	20 µl
Detection wavelength	238 nm
Flow rate	1 mL/min
Column temperature	25±2 °C

3.7. Linearity and range

The calibration curve was plotted, taking peak area and concentrations of standard solutions to establish linearity, utilizing selected conditions mentioned in **Table 4**. The calibration plot showed that biterin oil exhibited a linear response in the varying concentration range of 10-100 µg/mL, as shown in **Figure 6(B),** and the HPLC chromatogram of biterin oil is shown in **Figure 6(A)**. The correlation coefficient value of R^2 was less than 1.

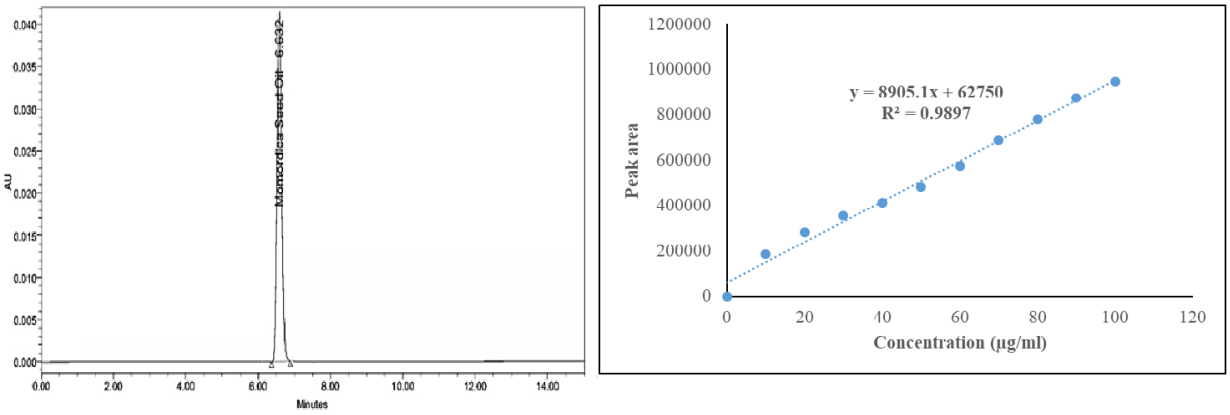


Fig. 5: (A) Chromatogram and (B) Calibration plot of Biterin oil

3.9. LOD and LOQ

The LOQ and LOD values were low (1.41 µg/mL and 3.4 µg/mL, respectively), suggesting that the method for estimating oil is sensitive.

3.10. Accuracy and recovery

In the accuracy method, we determined the recovery for LQC, MQC, and HQC. The observed %RSD value was less than 2%, as given in **Table 5**. The method developed for the estimation of biterin oil exhibited high accuracy, as the obtained results were within the limits.

Table 5. Accuracy data

Levels	Concentration of standard (µg/mL)	Number of sample injections	Mean area ± SD (n=6)	%RSD	% Recovery mean area ± SD
LQC	25	6	298485.8±994.5	0.33	105.8±0.42
MQC	50	6	483100.1±3543.4	0.73	94.3±0.78
HQC	75	6	724910.6667±4082.2	0.56	99.1±0.62

3.11. Precision

The LQC, MQC, and HQC, three quality control methods, were determined, and the %RSD was calculated for interday, intraday, and inter-analyst. The %RSD was estimated to be less than 2%, as given in **Table 6**, which suggested the results did not exceed the prescribed limits, showing the method was precise.

Table 6. Precision data

Levels	Conc. (µg/mL)	Interday precision	% RSD	Intraday precision	% RSD	Inter-analyst precision	% RSD
		Mean area ± SD (n=6)		Mean area ± SD (n=6)		A1 A2 A3	
LQC	25	298169.1±532.01	0.17	298387.1±747.2	0.25	297981.4±599.7	0.20
MQC	50	483108.8±3428.9	0.70	483314±2574.07	0.53	483593.9±3536.6	0.73
HQC	75	725615±4378.9	0.60	725615±4378.9	0.60	726530.5±4379.4	0.60

3.12. Robustness

The variation in the flow rate and wavelength was made to evaluate the robustness of the method. The %RSD was estimated to be less than 2%, showing that the process was robust. The values of robustness are shown in **Table 7.**

Table 7. Robustness data

Variable	Value	Number of sample injections	Mean area ± SD (n=6)	%RSD	Mean RT ± SD(n=6)	%RSD
Flow rate (mL/min)	0.6	6	482564.3±4454.2	0.92	4.7±0.05	1.18
	0.8	6	483875.8±3762.2	0.77	5.7±0.05	0.96
	1	6	483100.1±3543.4	0.73	6.5±0.03	0.54
Wavelength (nm)	233	6	456397.6±4800.5	1.05	6.45±0.05	0.84
	238	6	483100.1±3543.4	0.73	6.5±0.03	0.54
	243	6	470542.5±4563.31	0.96	6.4±0.11	1.82

4. CONCLUSION

The Soxhlet method of extraction yielded 55% oil, indicating high efficiency of the method. The FTIR absorption bands showed the existence of various long-chain fatty acids. The GCMS showed the presence of linoleic acid, which can be used for tissue regeneration and repair. Further, for the estimation of biterin oil, the RP-HPLC method was developed and validated. The developed method is advantageous for the estimation of extracted oil with a retention time of less than 10 mins. The development of the method utilized the Central Composite Design for optimization of chromatographic conditions. The responses obtained were calculated numerically and plotted. The effect of all independent variables on the dependent variables was evaluated. A well-defined peak was identified at 6.5 minutes as the retention time. The linearity was plotted over 10- 100 µg/mL, and a linear response of r^2=0.9897 was observed. The suggested method can be used successfully for the estimation of biterin oil in further studies.

Declaration of Competing Interest

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.

Funding

NA

Acknowledgement

The authors acknowledge the Central Instrumentation Facility (CIF), Lovely Professional University, Phagwara, Punjab, India, for providing the instrumental facilities and analytical support required to carry out this research.

Declaration of Generative AI and AI-assisted technologies in the writing process

This manuscript originated the idea and intellectual contribution from the authors. AI was used only for checking English grammar, correcting typos, and polishing writing style.

REFERENCES

1. Hussain A, Ramzan M, Altamimi MA, Khuroo T. HSPiP and QbD program–based analytical method development and validation to quantify ketoconazole in dermatokinetic study. AAPS PharmSciTech. 2023 Nov 14;24(8):231. https://doi.org/10.1208/s12249-023-02675-9
2. Bharate SS, Vishwakarma RA. Impact of preformulation on drug development. Expert opinion on drug delivery. 2013 Sep 1;10(9):1239-57. https://doi.org/10.1517/17425247.2013.783563
3. Bandopadhyay S, Bandyopadhyay N, Deb PK, Singh C, Tekade RK. Preformulation studies of drug substances, protein, and peptides: role in drug discovery and pharmaceutical product development. InDosage form design considerations 2018 Jan 1 (pp. 401-433). Academic Press. 10.1016/B978-0-12-814423-7.00012-5
4. Ahirwar K, Shukla R. Preformulation studies: a versatile tool in formulation design. InDrug formulation design 2023 Feb 23. IntechOpen. 10.5772/intechopen.110346 https://www.intechopen.com/chapters/86211
5. Yoshime LT, de Melo IL, Sattler JA, de Carvalho EB, Mancini-Filho J. Bitter gourd (Momordica charantia L.) seed oil as a naturally rich source of bioactive compounds for nutraceutical purposes. Nutrire. 2016 Sep 7;41(1):12. https://doi.org/10.1186/s41110-016-0013-y
6. Ahmad Z, Zamhuri KF, Yaacob A, Siong CH, Selvarajah M, Ismail A, Hakim MN. In vitro anti-diabetic activities and chemical analysis of polypeptide-k and oil isolated from seeds of Momordica charantia (bitter gourd). Molecules. 2012 Aug 10;17(8):9631-40. **https://doi.org/10.3390/molecules17089631**
7. Prashantha MA, Premachandra JK, Amarasinghe AD. Composition, physical properties and drying characteristics of seed oil of Momordica charantia cultivated in Sri Lanka. Journal of the American Oil Chemists' Society. 2009 Jan;86(1):27-32. https://doi.org/10.1007/s11746-008-1319-6
8. Thimmappa SP, Sudhir A, Rai KM, Deepakumari HN, Bhatt M. Potassium Iodate: A New Versatile Reagent to Determine the Iodine Value of Edible Oils. Lett. Appl. NanoBioScience. 2021;11(2):3537-41. https://doi.org/10.33263/LIANBS112.35373541
9. Lai Q, Xie Y, Wang M, Wang C, Sun K, Tan J. The complex refractive index of crude oils determined by the combined Brewster–transmission method. Infrared Physics & Technology. 2020 Dec

1;111:103515. https://doi.org/10.1016/j.infrared.2020.103515

10. Renuka R, Jeyanthi GP. FTIR and GCMS analysis of antidiabetic compounds in ethyl acetate seed extracts of Momordica charantia. Research Journal of Pharmacy and Technology. 2021 Dec 1;14(12):6705-9. 10.52711/0974-360X.2021.01158
11. Rashid MM, Akhter KN, Chowdhury JA, Hossen F, Hussain MS, Hossain MT. Characterization of phytoconstituents and evaluation of antimicrobial activity of silver-extract nanoparticles synthesized from Momordica charantia fruit extract. BMC complementary and alternative medicine. 2017 Jun 26;17(1):336. https://doi.org/10.1186/s12906-017-1843-8
12. Zotou A. An overview of recent advances in HPLC instrumentation. Central European Journal of Chemistry. 2012 Jun;10(3):554-69. https://doi.org/10.2478/s11532-011-0161-0
13. Solunke GJ, Das A. Development of a high-performance liquid chromatography (HPLC) method for analysis of charantin in bitter gourd (Momordica charantia) fruits. Crop Research. 2024;59(3and4):162-6. 10.31830/2454-1761.2024.CR-971
14. Zhan K, Ji X, Luo L. Recent progress in research on Momordica charantia polysaccharides: Extraction, purification, structural characteristics and bioactivities. Chemical and Biological Technologies in Agriculture. 2023 Jul 4;10(1):58. https://doi.org/10.1186/s40538-023-00433-4
15. Kumbhar M, Bagde S, Karpe M, Kadam V. HPLC method development and validation for estimation of eperisone hydrochloride from bulk and marketed formulation. International Journal of Pharmacy Research & Technology (IJPRT). 2019;9(1):6-14. https://www.ijprt.org/index.php/pub/article/view/99
16. Harish V, Almalki WH, Alshehri A, Alzahrani A, Gupta MM, Alzarea SI, Kazmi I, Gulati M, Tewari D, Gupta G, Dua K. Quality by design (QbD) based method for estimation of xanthohumol in bulk and solid lipid nanoparticles and validation. Molecules. 2023 Jan 4;28(2):472. **https://doi.org/10.3390/molecules28020472**
17. Annadi AM, El Zahar NM, El-Din A. Abdel-Sattar N, Mohamed EH, Mahmoud SA, Attia MS. Development and validation of molnupiravir assessment in bulk powder and pharmaceutical formulation by the RP-HPLC-UV method. RSC advances. 2022 Nov 30;12(53):34512-9. https://doi.org/10.1039/d2ra05066h
18. Naik M, Natarajan V, Rawson A, Rangarajan J, Manickam L. Extraction kinetics and quality evaluation of oil extracted from bitter gourd (Momardica charantia L.) seeds using emergent technologies. Lwt. 2021 Apr 1;140:110714. 10.1016/j.lwt.2020.110714
19. Singh TS, Verma TN. Biodiesel production from Momordica Charantia (L.): Extraction and engine characteristics. Energy. 2019 Dec 15;189:116198. https://doi.org/10.1016/j.energy.2019.116198
20. Ali AH. High-performance liquid chromatography (HPLC): A review. Ann. Adv. Chem. 2022 Jun 20;6(1):010-20. 10.29328/journal.aac.1001026