ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 19, Issue 3, 2026

Online - 2455-3891
Print - 0974-2441

Research Article

REVERSED PHASE-HIGH-PERFORMANCE-LIQUID CHROMATOGRAPHY METHOD FOR THE ESTIMATION OF MAGNOLOL IN SELF-NANO-EMULSIFYING DRUG DELIVERY SYSTEM

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Received: 30 November 2025, Revised and Accepted: 24 January 2026

ABSTRACT

Objectives: Reversed-phase high-performance liquid chromatography was used to produce a simple, accurate, and precise method for the estimation of Magnolol (MO) in self-nano-emulsifying drug delivery system.

Method: Analysis of this method was carried out using reverse phase Nucleodur C-18 column. For this study Mobile phase used was acetonitrile: H_2O (90:10% v/v), the flow rate was 1.0 mL/min, chromatogram of MO was determined at wavelength 290 nm, and sharp peak was obtained at retention time of 3.496 min. Validation of the developed method was done according to International Conference on HarmonizationQ2 (R1) guidelines.

Results: MO showed linearity at 2–10 µg/mL with R^2 of 0.9983. The % mean recovery was determined to be 95.01%, suggesting that the approach was accurate, and from the relative standard deviation which was found to be varying from 1.24-1.64% for intra-day and 0.53-1.96% for inter-day precision that is clearly <2%, indicating that the method was precise

. It was observed that the detection and quantification limits, that is, limit of quantification and limit of detection, were, respectively, 0.50 and 1.52 µg/mL. Finally, for the developed method, Robustness study was performed by doing small variations in pH, flow rate, ratio of mobile phase, and the result showed that the developed method was robust, as the percentage relative standard deviation and % recovery were under a specific limit.

Conclusion: This indicates that the method that was developed was accurate, linear, précised and robust. Furthermore, it can be used to estimate MO in Pharmaceutical formulations.

Keywords: Magnolol, Reversed phase high performance liquid chromatography, Validation, Self-nano-emulsifying drug delivery system.

 DOI: http://dx.doi.org/10.22159/ajpcr.2026v19i3.57667. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Magnolol (MO) is one of the polyphenolic lignin obtained from the bark of *Magnolia officinalis* [1]. Characteristic features of MO are that it is a white color powder compound bearing molecular weight 266.3 g/mol and has a melting point ranging from 101.5°C to 102°C [2]. MO is reported to have several pharmacological activities like anti-epileptic due to its effect on synaptic and presynaptic Gamma-Aminobutyric Acid (GABA) receptors [3], anti-inflammatory, analgesic [4], sedative-hypnotic [5], anti-oxidation, anti-micro-organism, anti-inflammation, anti-angiogenesis, anti-cancer, cardiovascular protection, neuroprotection, and lipolysis activity [6]. Despite having a wide range of pharmacological activities, its application is limited due to its poor bioavailability and solubility. Therefore, there is a requirement to develop a novel drug delivery system for MO so that its solubility and bioavailability can be improved [7]. As a result, researchers have developed nano-formulations such as micelles [8], micro-emulsion [9] in an effort to enhance the bioavailability and solubility of MO. It is necessary to develop an easy, sensitive, and repeatable method for quantifying these nano-formulations to evaluate them on various parameters.

There are a few techniques that have been available for the quantification of MO. Sheng *et al*. in 2014 reported pharmacokinetics of MO in combination with another compound using Ultra- Performance Liquid Chromatography- Tandem Mass Spectrometry (UPLC-MS/MS), which is sensitive and specific ultra-performance liquid chromatography/tandem mass spectroscopy [8]. Tsai and Chen, 1992 developed high-performance liquid chromatography (HPLC) with photodiode-array ultraviolet detection for the determination of the active principle of *M. officinalis* [10].

Tang *et al*., 2008 did a simultaneous estimation of eight major bioactive compounds by HPLC among them MO was also there, but individual method development of MO was not available, and also retention time reported was between 45 and 60 min [11]. Zhang *et al*., 2008 developed an HPLC method based on PDA detection for the estimation of 12 compounds, and among them, MO was also there and its retention time reported was 67 min [12]. Bio-analytical method was also reported by Lin *et al*., 2011, but detail information in the literature regarding analytical method development was not available [13]. However, in the present study, the analysis time of MO came out to be 3.496 min, which is significantly lower, thereby making it a key advancement over the previously reported methods. Hence, for analysis of MO, it is important to create and validate a simple approach that is an HPLC-based method and that can be used for MO formulations routine quality control. The main goal of this work was to develop a reverse phase method-HPLC (RP-HPLC) for the estimation of MO that was precise, repeatable, economical, and accurate.

MATERIALS AND METHODS

Materials

MO was received from the Xi'an Pincredit Biotech, China. Methanol, formic acid, ortho-phosphoric acid, tween 80, and acetonitrile (ACN) were received from Lobachemie Pvt. Ltd., Mumbai, India. Capmul MCM was procured from M/S Abitech Corp. (Mumbai, India). Transcutol P was procured from Gattefosse, Mumbai, as a gift sample. ACN HPLC

grade was procured from Merck, Mumbai, India. Throughout the study triple-distilled water was used.

Instruments

Instruments (RP-HPLC) Reversed Phase liquid chromatography system consisting of a binary pump (LC-20 AD); Prominence, Shimadzu, Japan), (DGU-20A5) a degasser unit, photodiode array detector (SPD-M20A; Shimadzu, Japan), Rheodyne injector with 20 µL sample injector loop, sonicator was used for degassing the mobile phase and Nucleodur C18 column with dimension 250 × 4.6 mm i.d., with particle size of 5 µm were used for development of analytical method. Chromatograms were captured with LC software. The study involved the use of a sonicator and an analytical balance (A×E 200; Shimadzu Analytical Pvt. Ltd., India).

Analytical method development

Chromatographic conditions

Selecting the correct chromatographic conditions is important for the development of a method. Different solvents, including orthophosphoric acid, methanol, ACN, formic acid, and triple-distilled water, were used in varying ratios, and the chromatogram was acquired at 290 nm. Various observations led to the selection of a mobile phase with a certain ratio, along with flow rate and retention time.

Formulation development

Self-nano-emulsifying drug delivery system (SNEDDS) consists of a homogeneous mixture of oil, surfactant, co-surfactant, and drug, respectively [14]. MO was mixed in the isotropic mixture of Capmul MCM, Transcutol P, and Tween 80. In the preliminary studies, 27 formulations were developed with varying ratios of 1:1, 1:2, and 2:1. The isotropic mixtures (1 mL) were prepared and vortexed for 15 min. Then, to the above mixture (1 mL), distilled water (500 mL) was added to form SNEDDS. Further, the mixture was stirred at 700–800 rpm for 7 min to achieve a spontaneous and stable self-emulsification zone, different ratios of selected oil, surfactant, and co-surfactant were mixed, and a pseudo-ternary phase diagram was plotted [15]. Then, using Stat Ease, USA software Box Behnken design was applied, and 17 SNEDDS prototype were developed and evaluated for PDI, drug loading, Zeta potential, self-emulsification time, and globule size.

Preparation of mobile phase

For analytical method development, mobile phase preparation was done by taking ACN and water in the ratio of (90:10), and pH of water was 7. The prepared mobile phase was filtered using 0.45µm membrane filter. Degassing of prepared mobile phase was done using ultra-sonicator.

Stock solution preparation

For analytical method development, stock solution (Solution A) was prepared in a standard volumetric flask, by accurately weighing 10 mg of MO and dissolving it in 10 mL of ACN to achieve the desired concentration of 1000 µg/mL. Then, 10 mL of solution A was taken out to achieve a final concentration of 100 µg/mL (Solution B). Further from solution B, 10 mL was withdrawn to obtain a final concentration of 10 µg/mL (Solution C). Then, finally, from solution C, further dilutions were prepared to obtain the concentration from 2 to 8 µg/mL.

Method validation

For validation of the developed method, International Council on Harmonization (ICH)Q2 (R1) guidelines were used. Based on these guidelines, three levels of quality control standards were prepared, which are Lower, medium, and high quantified concentrations (LQC, MQC, and HQC, respectively), and developed method was validated using validation parameters such as system suitability study, linearity, accuracy, precision, robustness, limit of quantification (LOQ), limit of detection (LOD).

System suitability study

System suitability assessment is a crucial component of analytical method validations because it ensures that the chromatographic system can conduct accurate and dependable investigations [16]. For the developed method, system suitability was determined on the basis of various factors such as peak purity index, height equivalent to theoretical plate, theoretical plate and tailing factor respectively. For evaluating the above given factors, a blank mobile phase (10 µg/mL) was injected, which was followed by six replicates [17,18]

Linearity and range

The ability to obtain test results that are directly proportional to the concentration of an analyte in a sample is what makes an analytical procedure unique [19,20]**.** The linearity studies of MO were performed by plotting different concentrations (2,4,6,8,10 µg/mL) and were injected 6 times each, and the regression equation was recorded.

Accuracy study

An analytical method's accuracy is expressed as the degree to which the predicted value and the observed value are close [21]. Based on absolute recovery, an accuracy study was performed. This study was carried out at three levels, which had been referred to as LQC, MQC, and HQC, respectively, and these values were 80, 100, and 120% of the mid concentration, which is 6 µg/mL of the calibration curve. Six injections of the standard solutions for each of the three levels were made, and the response mean values were noted. Percentage absolute recovery was calculated from the formula given below [22].

$$\text{Percentage recovery} = \frac{\text{Actual concentration recovered}}{\text{Theoretical concentration}} \times 100 \quad (1)$$

Precision

A precision study involves two steps, that is, intraday and intermediate. In intraday six injections of LQC (80%), MQC (100%), HQC (120%) were injected on the same day under the same experimental conditions. Intermediate involves two steps inter-day and inter-analyst. In inter-day, on three different days, six injections of LQC, MQC, and HQC were injected. In inter-analyst study, three different analysts injected six injections of LQC, MQC, and HQC. On the basis of the response obtained, the mean and percentage relative standard deviation were calculated [18].

Robustness

Robustness study of the developed method was evaluated by purposely altering the experimental conditions. The method must be reliable enough to endure small changes during repeated analysis of sample. Study was conducted by altering the ratio of mobile phase ACN: water (88:12, 90:10, and 92:08 v/v), pH (6.8, 7, 7.2), and flow rate (0.8, 1, and 1.2 mL/min). Medium concentration 6 µg/mL was injected 6 times, and their effect on peak area, retention time, and percentage recovery was noted [23].

Estimation of LOD and LOQ

LOD is a measurement of minimum analyte concentration that provides a measurable response, while LOQ is the lowest concentration that can be quantified precisely and accurately [22]. For the determination of LOD and LOQ, different methods were used, such as the calibration curve method, signal-to-noise ratio, and visual evaluation method. In this method calibration curve method was used using the equations given below [17].

$$\text{LOD} = 3.3 \times \text{standard deviation/slope} \quad (2)$$

$$\text{LOQ} = 10 \times \text{standard deviation/slope} \quad (3)$$

Where, as
Standard deviation of calibration curve is denoted as σ
Slope of calibration curve is denoted as S

Application of HPLC method in solubility and drug loading estimation

Determination of drug solubility

For selection of suitable components (oil, surfactant and co-surfactant) for the formulation of SNEDDS, the solubility studies was performed for raw MO in oils (peanut oil, eucalyptus oil, cotton seed oil, olive oil, Capmul MCM, Labrafil M2125 CS, Capryol 90, Isopropyl Myristate, Capryol PGMC, surfactants (Labrafac lipophile WL1349, Labrafil M1944 CS, Tween 80, Tween 20, Tween 60, Span 80, and Labrasol, Labrafac PG,) and co-surfactants (PEG 400, Transcutol P, Transcutol HP, Lauroglycol FCC, Maisine CC, Propylene Glycol, Lauroglycol 90, and Plurol oleique) respectively. To 1 mL of each oil, surfactant, and co-surfactant, an excess amount of drug was taken in a clean glass vial and vortexed (CM 101 CYCLO MIXER, REMI, India) for 2 min for proper mixing of MO with the vehicle. The vials were stoppered and agitated for 72h at 37±0.2°C in a shaking water bath. On equilibration, all the samples were centrifuged after 72 h in (REMI CM-12 PLUS, India) at 10000 g for 20 min for the removal of the undissolved MO from saturated solutions. The supernatants were accurately measured and appropriately diluted with a suitable solvent, and the amount of drug was estimated by HPLC [24,25].

Estimation of drug loading in SNEDDS

SNEDDS was prepared by taking 10 mg of MO in 1 mL mixture of selected oil, surfactant, and co-surfactant. Then the formulations were diluted up to 500 mL with triple-distilled water on a magnetic stirrer at 700–800 rpm at room temperature. Drug loading in SNEDDS formulation was evaluated by using HPLC. Formulation sample was filtered using a syringe filter and was injected into HPLC system to analyze the MO peaks. Percentage drug loading was calculated using the following equation [26]:

$$\%\text{drug loading} = \frac{\text{Concentration of drug quantified in SNEDDS}}{\text{Total amount of polymer added}} \times 100$$

RESULTS AND DISCUSSION

Mobile phase selection for estimation of MO

Mobile phase consist of different ratios has been used, and their results were analyzed. It was observed that when methanol: water (80:20 v/v pH 3) was used as the mobile phase chromatogram of MO was not appropriate. When mobile phase used was ACN:0.2% Ortho-phosphoric acid (90:10) (pH 4), then peak observed was broader with a shoulder. Similarly, like this many trials conducted with ACN: Phosphate buffer (different pH range), methanol: Ortho phosphoric acid, but the peak obtained was broader with a shoulder and tailing, which is not acceptable. When ACN and water were used in the ratio of 90:10 v/v (pH of water 7) at a flow rate of 1 mL/min then as a result a sharp and single peak was obtained at retention time of 3.496 min. As a result, this mobile phase combination was considered as final for the purpose of validation. The obtained result was shown in (Fig. 1).

Method validation

System suitability study

Suitability test was performed to confirm that the two parameters, that is, chromatographic system reproducibility and solution, are sufficient for the analysis to be performed. Results of six injections of blank

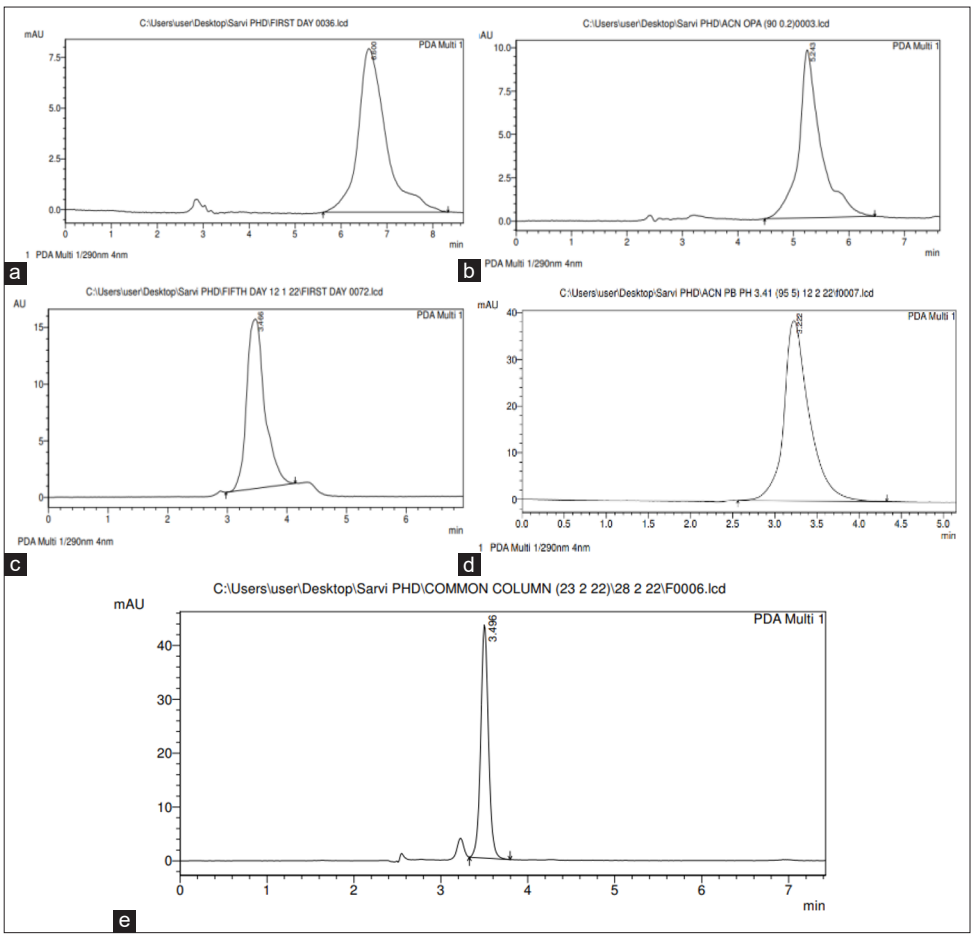

Fig. 1. Chromatogram of Magnolol with different mobile phases, (a) methanol: water (80:20 v/v (pH3), (b) ACN:0.2% Ortho-phosphoric acid (OPA) (90:10 v/v), (c) Methanol:0.1% OPA (95:5 v/v), (d) ACN: phosphate buffer (0.01M/pH3.41) 95:5 v/v, (e) ACN: water (90:10v/v) (pH 7)

solution were found to be in an acceptable range, and system suitability data are shown in Table 1.

Linearity and range

The linearity studies of MO were performed by plotting different concentrations of the standard solution against their respective area, and the composition of the mobile phase ratio was 90:10, v/v (ACN: water). 1.0 mL/min flow rate and wavelength used was 290 nm to detect a chromatogram. It was observed that the drug shows linearity between the range of 2 and 10 µg/mL as shown in (Fig. 2).

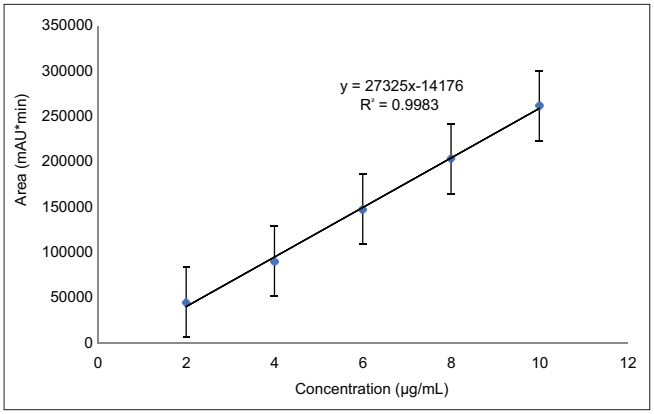

Fig. 2: Magnolol's calibration curve

Table 1: Results of Magnolol suitability study

Parameters	Value	Limits
HETP	24.64±0.27	depends upon theoretical plate
Theoretical plate	6089.57±68.51	More than 2000
Theoretical plate/meter	40597.25±456.76	More than 20000
Tailing factor	1.13±0.002	Less than 2
Peak purity index	0.999	More than 0.5

*HETP: Height equivalent to a theoretical plate, *Data are expressed as mean±standard deviation; n=6

Accuracy

Accuracy can be expressed in terms of percentage recovery as the closeness of the test result to that of true value. The study was performed at three different levels, such as LQC, MQC, and HQC, and was analyzed in triplicate. In pre-analyzed sample solution, standard drug solution was added and % recovery and % mean recovery were measured, which came under the specific limit. The obtained results are shown in (Table 2).

Precision study

During precision study developed method was evaluated on the basis of intermediate precision and repeatability. In repeatability studies under similar experimental conditions, % RSD was calculated for six values of three different levels at same day of sample preparation, the next day, and by three different analysts. Obtained results were presented in Table 3, and it was found that % relative standard deviation was varying from 0.53 to 1.96. This result clearly revealed that the method was precise.

Robustness study

Robustness of new method was examined through little alteration in experimental condition such as by doing changes in the mobile phase ratio that is ACN: water (88:12, 90:10, and 92:08 v/v), pH (6.8,7,7.2) and flow rate (0.8, 1, and 1.2 mL/min) respectively, on retention time, peak area and % recovery of the sample. Peak area's % RSD, RT, and percentage recovery after a small change in pH were found 0.83,3.6,111.01, respectively. Similarly, %RSD after doing a small change in rate of flow and mobile phase ratio was calculated for area of peak, RT, and percentage recovery, and it was varying from 0.66-1.69, which is under the specified limit of 2% (Table 4).

Estimation of LOD and LOQ

Standard dilution values were found to be 0.50 and 1.52 µg/mL, respectively. This clearly indicates that minimum concentration which

Table 2: Accuracy studies results

Levels	Standard solution (µg/mL)	Sample solution (µg/mL)	Observed concentration (µg/mL)	Percentage recovery (%)	Mean recovery (%)
LQC	4.8	6	4.49	93.54	95.01
HQC	6.0	6	5.94	99.0	
MQC	7.2	6	6.66	92.5	

*HQC: High quantified concentration, LQC: Lower quantified concentration, MQC: Medium quantified concentration. *Data are expressed as mean±standard deviation; n=6

Table 3: Precision study results

Parameters	Levels	Concentration (µg/m L)	Analytical responses (area), injections						Mean (*n=6)	SD	%RSD
			1	2	3	4	5	6			
Repeatability (intraday precision)											
	LQC	4.8	99207	95911	99502	97991	100033	100985	98938.16	1624.76	1.64
	MQC	6	150303	150620	146427	146379	146518	149190	148239.5	1850.15	1.24
	HQC	7.2	162144	161376	159932	157688	161855	156840	159972.5	2051.91	1.28
Intermediate precision (inter-day)											
Day 1	LQC	4.8	110023	111345	109869	110387	109925	111180	110454.83	596.27	0.53
	MQC	6	144430	143975	148227	149970	151417	148862	147813.5	2741.29	1.85
	HQC	7.2	169026	170011	172596	171005	169982	170054	170445.6	1118.74	0.65
Day 2	LQC	4.8	100569	100880	102249	100680	101893	105236	101917.83	1609.82	1.57
	MQC	6	156853	160501	158141	156193	155254	154540	156913.66	1970.07	1.25
	HQC	7.2	166841	164918	165818	173890	165043	170521	167838.5	3297.42	1.96
Day 3	LQ C	4.8	116858	115716	117445	118475	118385	114349	116871.33	1465.67	1.25
	MQ C	6	166311	164323	170517	166654	167917	167322	167174	1866.05	1.11
	HQC	7.2	173844	177522	174092	177328	177794	171974	175425.66	229.23	1.27

*RSD: Relative standard deviation. *Data are expressed as mean±standard deviation; n=6

Table 4: Results of robustness study

Various parameters	Value	Sample concentration (µg/mL)	Peak area (mean±SD) (*n=6)	Average of peak areas values (*n=3)	RT (mean±SD) (*n=6)	Average of retention times (*n=3)	% Recovery (mean±SD) (*n=3)	Average of % recoveries (*n=3)
pH	6.8	6	166292.8±2730.60	164597.77	3.60±0.06	3.6	110.07±1.66	111.01
	7	6	164570.5±5036.02	SD=1372.99	3.63±0.01	SD=0.02	112.97±0.29	SD=1.38
	7.2	6	162930±4562.05	%RSD=0.83	3.58±0.01	%RSD=0.66	110.01±2.77	%RSD=1.24
Flow rate	0.8	6	142828.2±2964.71	143953.53	3.2995±0.003	3.26	102.99±1.27	101.2
	1	6	147067.7±3474.84	SD=2230.08	3.250±0.01	SD=0.02	100.26±2.12	SD=1.26
	1.2	6	139964.7±4811.49	%RSD=1.54	3.224±0.02	%RSD=0.66	100.35±2.93	%RSD=1.25
Mobile phase ratio (A: B)	88:12	6	165067±3118.89	166509.7	3.69±0.003	3.61	109.32±1.90	112.05
	90:10	6	165396.5±57554.87	SD=1812.34	3.54±0.004	SD=0.02	112.64±1.18	SD=2.03
	92:08	6	169065.7±1262.90	%RSD: 1.09	3.61±0.008	%RSD=1.69	114.21±0.77	%RSD=1.81

*n: Number of replicates of study conducted, SD: Standard deviation, RSD: Relative standard deviation. *For each robustness parameter (pH, flow rate, mobile phase ratio), three different conditions were tested. For each condition, six injections (n=6) were made. *The column 'Average of... (n=3)' represents the mean of the three condition-specific average values

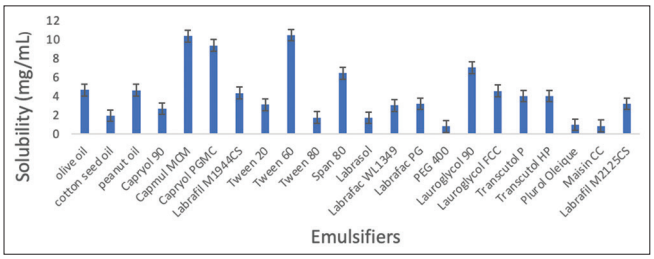

Fig. 3: Magnolol's solubility studies in oil, surfactant and co-surfactant

is sensed by the developed method is 0.50 µg/mL, as the minimum concentration which is accurately measured by the developed method is 1.52 µg/mL. The concentration range selected for the preparation of the standard calibration graph is 2–10 µg/mL, which is validated by the above results.

Application of HPLC method in estimation of drug solubility

Solubility of MO

Solubility study of MO was done in various oil, surfactant, and co-surfactant. The solubility was found highest in Capmul MCM, Capryol PGMC, Tween 60, Tween 80, and Lauroglycol 90, Transcutol P in oil, surfactant, and co-surfactant, respectively (Fig. 3). This study helps determining key components for SNEDDS formulations.

Estimation of drug loading

The drug loading was found to be 9.45±4.97 in SNEDDS, as the total drug load was 10 mg, and the reason for drug loss in SNEEDS may be due to drug precipitation, drug degradation, interaction with excipients, enzymatic metabolism [27,28].

CONCLUSION

The developed analytical method was verified on the basis of precision, accuracy, robustness, and LOD and LOQ. The results of all these parameters were reported within the specified limit of ICH, indicating that the devised method can be used for evaluating MO because it was precise, accurate, sensitive, and simple. Hence, this method can be used to estimate MO in diverse pharmaceutical formulations.

ACKNOWLEDGMENTS

The authors are thankful to Gattefosse in India for giving emulsifier gift samples.

AUTHORS' CONTRIBUTIONS

Sarvi Yadav: Conceptualization, Writing – original draft, visualization, Review, Editing, Data curation. Narendra Kumar Pandey: Project administration, Supervision, Resources, Visualization, Data curation, Review and Editing. Abhinav Anand: Resources, Formal analysis. Bimlesh Kumar: Supervision, Data curation, Review.

CONFLICTS OF INTERESTS

No conflicts of interest were declared by the authors.

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