QUANTITATIVE ESTIMATION OF 6-GINGEROL, *E*-GUGGULSTERONE AND *Z*-GUGGULSTERONE IN A FIXED DOSE COMBINATION NANOEMULGEL BY RP-HPLC

Anita D. Chando[a], Vivek Basudkar[b], Sankalp Gharat[b], Munira Momin[b,c*] and Tabassum Khan[d]

(Received 10 October 2022) (Accepted 13 June 2023)

ABSTRACT

Polyherbal formulations have proved to be efficacious for the therapeutic treatment of various diseases, However, the development of validated robust analytical methods for quantification is a major challenge. The aim of this project was to develop a simple analytical method for the quantification of 6-gingerol (6-GIN), *E*-guggulsterone (*E*-GGS) and *Z*-guggulsterone (*Z*-GGS) in nanoemulsion based gel using reverse phase high performance liquid chromatography (RP-HPLC). 6-GIN, *E*-GGS and *Z*-GGS were quantified using acetonitrile: water: methanol (70:20:10 V/V/V) as the mobile phase at 1.0 mL min^{-1} flow rate with photodiode array detection. The developed method was validated for linearity, accuracy, precision, specificity and robustness as per ICH Q2 (R1) guidelines. The drug content of the three actives in the developed nanoemulgel was found to be between 90% to 110% w/w. The developed analytical method is simple and can be used for quantification of 6-GIN, *E*-GGS and *Z*-GGS in fixed dose product containing these actives.

Keywords: Nanoemulsion,6-Gingerol, *E*-guggulsterone and *Z*-guggulsterone, high pressure liquid chromatography

INTRODUCTION

Herbal formulations provide many alternative therapeutic approaches for the treatment of various diseases. These often contain two or more chemical compounds which play a significant role in treatment of acute and chronic conditions[1]. Hence, it becomes essential to quantify the analyte of interest and to determine its content in formulation and plasma. However, the quantification of the same poses a major challenge due to the complexity of separation. High performance liquid chromatography (HPLC) is among the widely used methods for separation and quantification of chemical compounds.

Rheumatoid arthritis (RA) is a chronic immune-mediated disorder associated with persistent inflammation, swelling and stiffness of joints[2,3]. 1.3 million American population has RA, 41 in each 100,000 human population have RA. Diagnosis of the disease can be done in the initial three months to two years from the onset of disease[2]. Topical application of drugs for therapeutic treatment of RA have gained the interest of scientists, due to fewer side effects as well as ease of administration. Concerns about the adverse effects and economic burden of topical dosages containing conventional drugs for RA has led to the use of natural remedies in treatment of RA.

Ginger oleoresin (GOR) contains shogaol and gingerols, extracted from ginger rhizomes *(Zingiber officinale)* and inhibit and cyclooxygenase-2 (COX-2), translating to anti-inflammatory activity. 6-Gingerol is reported to inhibit prostaglandin (PG) and leukotriene biosynthesis *via* suppression of 5-lipoxygenase (5-LOX) and PG synthetase[4–6]. Further, it inhibits pro-inflammatory cytokines synthesis such as IL-1β, IL-6, and TNF-α, resulting in anti-inflammatory activity[7]. Chemically, 6-Gingerol (6-GIN) is 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one. Lipid guggul extract (LGE) contains *E*- and *Z*- guggulsterones and is obtained from mukul myrrh *(Commiphora mukul).* It is reported for

[a] Regulatory Affairs Department, Madison Ortho, Powai, Mumbai - 400 076, Maharashtra, India
[b] Department of Pharmaceutics, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai - 400 056, Maharashtra, India
[c] SVKM's Shri C. B. Patel Research Centre for Chemistry and Biological Sciences, Vile Parle (West), Mumbai - 400 056, Maharashtra, India
[d] Department of Pharmaceutical Chemistry, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai - 400 056, Maharashtra, India
* For Correspondence: E-mail: munira_momin@yahoo.com

https://doi.org/10.53879/id.60.07.13722

anti-inflammatory activity by ameliorating the levels of IL-6, TNF-α, IL-1β, NO, IL-12 and IFN-γ[8]. *E*- and *Z*- guggulsterones decrease and pro-inflammatory cytokines and suppress cyclooxygenase-2 (COX-2) mRNA levels, thereby demonstrating anti-inflammatory and anti-arthritic activity. Chemically, *E*-guggulsterone *(E*-GGS*)* and *Z*-guggulsterone *(Z*-GGS*)* are 4, 17(20)-pregnadiene-3, 16-diones[9,10]. The chemical structures of 6-GIN, *E*-GGS and *Z*-GGS are illustrated in Fig. 1.

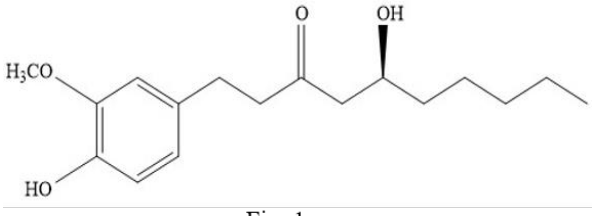

Fig. 1a

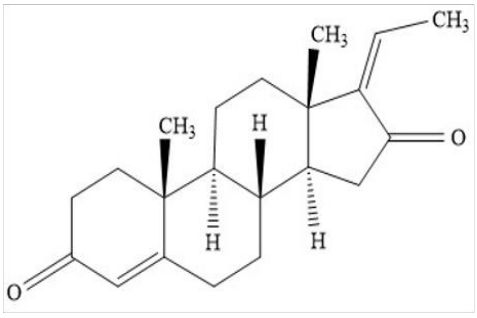

Fig. 1b

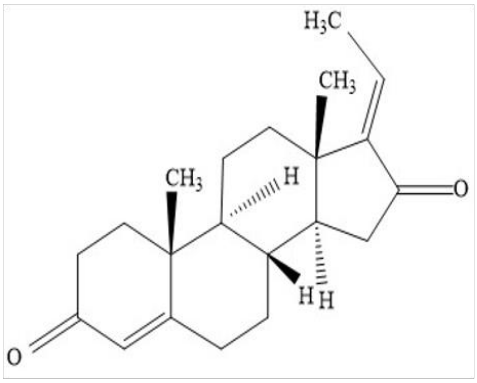

Fig. 1c

Fig. 1: Chemical structure of a) 6-gingerol, b) *E*-guggulsterone and c) *Z*-guggulsterone

Ginger oleoresin (GOR) and lipid guggul extract (LGE) are lipophilic in nature, hence delivering them as a nanoemulsion can potentially increase their efficacy in the management of pain and inflammation associated within RA. Literature study reports that different methods like RP-HPLC[11], LC-MS[12,13] and HPLC–UV–ES-MS[14] have been used for the estimation of the above constituents individually in plasma[15] and extracts[16]. The quantification of all three phytoconstituents in a pharmaceutical dosage has not been reported till date. In the present study, the authors have tried to develop a simple, specific and accurate method for the quantitative estimation of 6-GIN, *E*-GGS and *Z*-GGS in nanoemulgel dosage form developed for management of RA.

MATERIALS AND METHODS

Ginger oleoresin and lipid guggul extract were offered as gift samples from Sunpure Extracts Pvt. Ltd., New Delhi and Arjuna Naturals Pvt. Ltd., Kerala, respectively. 6-Gingerol (HPLC purity 96.8%), E-guggulsterone (HPLC purity 99.6%) and *Z*-guggulsterone (HPLC purity 97.6%) working reference materials (*E*-GGS and *Z*-GGS) were obtained as individual working reference materials and were procured from Natural Remedies Pvt. Ltd., Kerala. Methanol and acetonitrile (HPLC grade) were bought from SD Fine Chemicals Ltd., All other solvents and chemicals used in this project were of analytical grade. 6-GIN, *E*-GGS and *Z*-GGS loaded nanoemulgel was formulated in the lab for the management of RA.

HPLC instrumentation and chromatographic conditions

Agilent HPLC system (Infinity 1260) with auto sampler and photo diode array (PDA) detector was used. The chromatography separation of 6-GIN, *E*-GG*S* and *Z*-GGS was performed on C18 column (250x 4.5mm, 5µm) at 37±0. 02 °C. Acetonitrile (ACN): water (H_2O): methanol (MeOH) in the ratio (70:20:10 V/V/V) was used as mobile phase with 1.0 mL min^{-1} flow rate. The phytoconstituents peaks were detected at 235 nm using 10 µL injection volume.

Preparation of working reference material stock solution

5 mg each of 6-GIN, *E*-GGS and *Z*-GGS was dissolved in 5 mL methanol and sonicated to prepare three stock solutions of the working reference material (1000 µg mL^{-1}).

Method development

The further dilution of stock solution was done to get 10 µg mL^{-1} of 6-GIN, *E*-GGS and *Z*-GGS each using the mobile phase. Further, the samples were loaded in autosampler for recording the chromatograms. The chromatographic condition was optimized to obtain well resolved peaks in a reasonable analytical run time.

Method validation

The developed analytical method was validated for the following parameters according to ICH Q2 (R1) guidelines.

System suitability

System suitability was performed by loading six injections containing 30 µg mL^{-1} of G-GIN, *E*-GGS and *Z*-GGS each. The number of theoretical plates, retention time, resolution and asymmetry factor of the chromatographic peaks were determined.

Linearity and range

Linearity studies were performed using a calibration curve of 10-100 µg mL^{-1} of 6-GIN, *E-GGS* and *Z-GGS* each in methanol. The graph of peak area was plotted against corresponding concentrations in µg mL^{-1} and subjected to linear regression analysis to obtain the slope and y-intercept.

Specificity

Specificity was carried out by dissolving 500 mg of placebo nanoemulsion containing castor oil, fenugreek oil, Capmul MCM, oleic acid, Kolliphor ELP, Tween 80 and propylene glycol in 10 mL methanol. The working solution was then prepared by diluting the above-mentioned stock solution using the mobile phase.

Precision

Precision study was carried out by performing repeatability, intermediate precision and reproducibility. Repeatability was done by the preparation of minimum six different samples (n=6) of 30 µg mL^{-1} of 6-GIN, *E*-GGS, *Z*-GGS each which were analysed. Intermediate precision was performed at two different levels (intra-day and inter-day study). Intra-day precision was performed by preparing 30 µg mL^{-1} of 6-GIN, *E*-GGS and *Z*-GGS, respectively and analysing (n=6) at 3 different time intervals on the same day. Inter-day precision was determined by analysing the samples (n=6) for three successive days.

Accuracy

Accuracy study was performed by spiking working reference material solution of 6-GIN, *E*-GGS and *Z*-GGS to the placebo. Concentrations of 6-GIN, *E*-GGS and *Z*-GGS corresponding to 80%, 100%, and 120% were spiked and percent recovery was calculated. The study was carried out in triplicate and mean %RSD was calculated.

LOD and LOQ

The limit of detection (LOD) is used to determine the lowest detectable concentration while LOQ is the minimum quantifiable concentration of the analyte. The following equations were used to calculate LOD and LOQ:

$$Limit\ of\ quantification\ (LOQ) = \frac{10\text{x}\ \sigma}{Slope}$$

$$Limit\ of\ detection(LOD) = \frac{3.3\ \text{x}\ \sigma}{Slope}$$

where, σ is the standard deviation of the response.

Robustness

Robustness was performed by changing the temperature and flow rate. Parameters like tailing factor, resolution, asymmetric factor and number of theoretical plates were determined. The following changes were made to evaluate the robustness.

Variation in flow rate*:* The reliability of the standard was measured by altering the flow rate from 0.9 mL min^{-1} to 1.1 mL min^{-1}.

Variation in temperature: The reliability of the standard was measured by changing the temperature of column from 36.5 °C to 37.5 °C.

Drug content determination

The concentration of 6-GIN, *E*-GGS and *Z*-GGS in the NE gel was determined by using the developed method. 1g of NE gel was diluted to 10 mL methanol. After suitable dilutions with mobile phase (ACN: methanol: water-70:10:20), the amounts of 6-GIN, *E*-GGS and *Z*-GGS in the nanoemugel were determined.

In vitro diffusion study

The *in vitro* drug diffusion of the optimized gel was performed using Franz Diffusion apparatus on nylon

membrane (0.45µm). The membrane was pre-soaked in the release media overnight at ambient temperature. The receptor compartment was filled with phosphate buffer pH 7.4: ethanol (1:1) + 3 %w/w Tween 80 (release media) in isothermal condition (37 °C±2 °C) and stirred at 100 rpm on a magnetic stirrer. Aliquots were removed at hourly time intervals (1-12 h. and at 24 h) and analyzed for the content of three actives by the developed analytical method. The samples were replenished with fresh media after removing the aliquots. A graph of % cumulative drug release was plotted against the time to study the release of the actives[17].

Stability studies

Stability studies of the developed gel were conducted at different stability conditions – 5 °C ± 3°C (3 months), long term stability studies: 25 °C ± 2 °C/ 60% RH ± 5% RH (3 months), accelerated stability conditions 40°C ± 2°C/ 75% RH ± 5% RH (3 months) as per ICH Q1A (R2) guidelines.

Statistical analysis

All values are expressed as mean ± S.D. of determinations. Statistical differences were analysed by using one-way analysis of variance (ANOVA). Stability study drug content assays were analysed using the student's t-test at 95% confidence interval. For all statistical analyses, Microsoft Excel 2013 was used. Values of $p < 0.05$ were considered significant in all tests.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

An HPLC based analytical method for diffusion studies and assay was developed using ACN: H_2O: MeOH in the ratio 70:20:10 V/V/V as the mobile phase, and a 1.0 mL min^{-1} flow rate using C-18 (250 x 4.5mm, 5µm) 37 °C ± 0.2 °C was found to be optimum and yielded reproducible peaks. Chromatographic conditions were varied to obtain a chromatogram with best peak shape and good resolution. Table I highlights the optimization

Table I: Optimization of chromatographic conditions

Sr. No.	Chromatographic condition	Observation	Conclusion
1	Column: C18 (250 x 4.5mm, 5µm) PDA wavelength 235nm ACN: water 50:50 (V/V) Run time 35mins	Peak splitting was observed with no sharp peak	Method was not accepted
2	Column: C18 (250 x 4.5 mm, 5µm) PDA wavelength 235 nm ACN: water 60:40 (V/V) Run time 20 mins	Dual peaks were observed for all three actives.	Method was not accepted
3	Column: C18 (250 x 4.5mm, 5µm) PDA wavelength 235nm ACN: water 70:30 (V/V) Run time 20mins	Peak resolution between 2nd and 3rd peak was less than 2%	Method was not accepted
4	Column: C18 (250 x 4.5mm, 5µm) PDA wavelength 235nm ACN: MeOH: water 70:10:20 (V/V/V) Run time: 10 mins	Well resolved and high intensity peaks achieved. Selected mobile phase for further study based the run time and peak resolution.	Method was used for validation
5	Column: C18 (250 x 4.5mm, 5µm) PDA wavelength 235nm ACN: MeOH: water 70:20:10 (V/V/V) Run time: 10 mins	Peak resolution between 2nd and 3rd peak merges and fronting was observed	Method was not accepted

of chromatographic conditions. Initially, water and ACN were used in varying ratios and later methanol was added to obtain the desired chromatogram. The retention times of 6-GIN, *E*-GGS and *Z*-GGS were3.5min ± 0.5, 5.32min ± 0.5, 6.23min ±0.6, respectively at 235nm (Fig. 2).

Linearity and range

Linearity was performed by preparing 6 different dilutions of 6-GIN, *E*-GGS and *Z*-GGS in the range 10-100 µg mL^{-1} in triplicate. The correlation coefficient (R^2) of all the phytoconstituents were found to be 0.99. The R^2

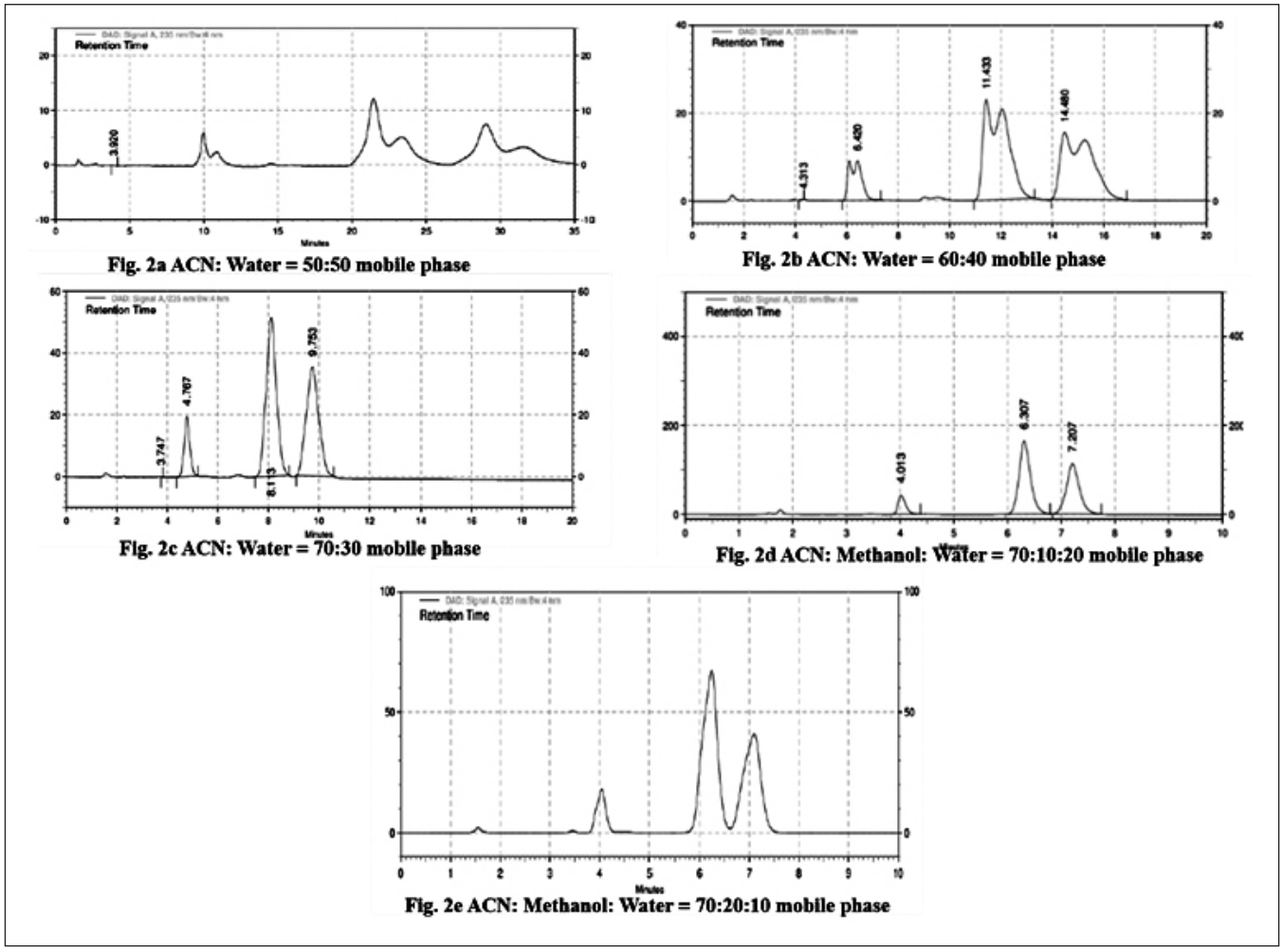

Fig. 2: a,b,c,d and e show different chromatograms condition by varying the ratio of solvents in mobile phase and different run times

Table II: Linearity study of 6-gingerol, *E*-guggulsterone and *Z*-guggulsterone

Parameter	6-gingerol	*E*-guggulsterone	*Z*-guggulsterone
Linearity range	10-100 µg mL^{-1}	10-100 µg mL^{-1}	10-100 µg mL^{-1}
Regression equation	y=3880.5x-3826.7	y=284.55x-1780.1	y=493.92x-4848.7
Correlation coefficient	0.9987	0.9912	0.9941
Slope	3880.5	284.55	493.92
Intercept	3826.7	1780.1	4848.7

Table III: System suitability study of 6-gingerol, *E*-guggulsterone and *Z*-guggulsterone

Parameter	6-gingerol	*E*-guggulsterone	*Z*-guggulsterone	Acceptance criteria
Retention time	3.99	6.14	6.98	-
Resolution	-	8.88	2.61	R_S>2
No. of theoretical plates	7408.67	6828.17	6358.83	N > 2000
Asymmetry factor	1.08	0.92	0.87	A_s< 2

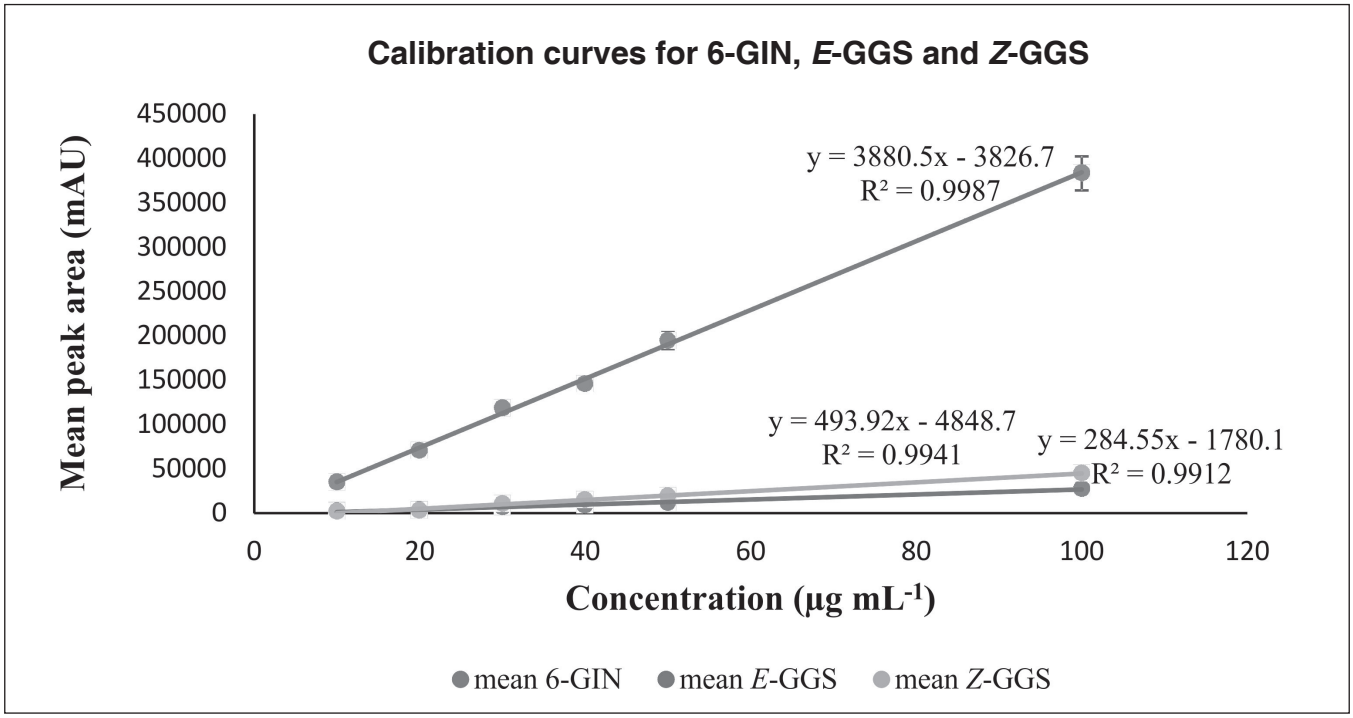

Fig. 3 : Calibration curve of 6-gingerol, *E*-guggulsterone and *Z*-guggulsterone

Table IV: Precision study of 6-gingerol, *E*-guggulsterone and *Z*-guggulsterone

Parameter	6-gingerol		*E*-guggulsterone		*Z*-guggulsterone	
	Mean area mAU	% RSD	Mean area mAU	% RSD	Mean area mAU	% RSD
Repeatability (n=6)	115610.33± 1965.11	1.69	6365.5±68.18	1.07	10467.67±144.29	1.37
Inter-day precision (n=6)	124531.249± 1875.11	0.47	6478.5±58.15	0.25	11348.549±132.189	0.51
Intra-day precision(n=6)	118158.333± 891.89	0.75	6717.333±78.26	1.16	10508±188.42	1.79

and %RSD were within the limits. The calibration curve graph and data are depicted in Fig. 3 and Table II.

System suitability

The results of system suitability are shown in Table III. All the parameters of the system suitability like theoretical plates, tailing factor and resolution demonstrated that the developed method was suitable for quantitative analysis.

Specificity

The mobile phase did not show interference with any of the analytes at their respective retention times, indicating that the optimized method is specific.

Precision

The results of repeatability and intermediate precision are illustrated in Table IV. The % RSD of 6-GIN, *E*-GGS and *Z*-GGS was below 2% that validates the reproducibility of the developed method.

Accuracy

The mean recovery of 6-GIN, *E*-GGS and *Z*-GGS was between 97% to 103% at three different levels. The results for recovery at 80, 100, and 120% were found to be within the predefined range. As a result, the methodology was proved to be reliable, and the results are displayed in Table V.

Table V: Accuracy study of 6-gingerol, *E*-guggulsterone and *Z*-guggulsterone

	Level	Std amount (µg mL^{-1})	Amount added (µg mL^{-1})	Total amount (µg mL^{-1})	Amount recovered (µg mL^{-1})	% Recovery	% RSD
6-gingerol	80%	30	24	54	53.12	98.38	1.17
	100%	30	30	60	61.40	102.34	0.63
	120%	30	36	66	67.21	101.84	1.19
E-guggulsterone	80%	30	24	54	53.05	98.24	0.49
	100%	30	30	60	59.38	98.96	0.02
	120%	30	36	66	67.123	101.71	1.13
Z-guggulsterone	80%	30	24	54	54.42	100.77	1.49
	100%	30	30	60	60.09	100.16	0.40
	120%	30	36	66	65.4	99.09	1.21

Table VI: Robustness study of 6-gingerol, *E*-guggulsterone and *Z*-guggulsterone

Variable	Level (±)	6-gingerol		*E*-guggulsterone		*Z*- guggulsterone	
		Mean area	% RSD	Mean area	% RSD	Mean area	% RSD
Flow rate	0.9 mL	119118.33 ± 672.29	0.56	6670.67 ± 116.13	1.74	10126.33 ± 111.73	1.10
	1.0 mL	118426.50		6781.48		10411.67	1.21
	1.1 mL	116358.67 ± 741.03	0.63	6393.33 ± 127.33	1.99	10599.33 ±59.18	0.55
Temperature	36.5 °C	114248±1448.01	1.26	6780.333±70.06	1.03	10652.667±193.42	1.81
	37 °C	118426.50		6781.48		10411.67	1.21
	37.5 °C	117295.67±318.72	0.27	6578±52.37	0.79	10581.33±72.27	0.68

Table VII: *In vitro* drug release study of 6-GIN, *E*-GGS and *Z*-GGS in nanoemulgel

Time (h)	% Drug release of 6-GIN	% Drug release of *E*-GGS	% Drug release of *Z*-GGS
0	0	0	0
1	03.22±1.23	02.41±1.33	05.07±0.41
2	08.40±0.87	05.55±2.87	10.95±1.45
3	14.83±2.48	9.29±1.22	17.80±1.78
4	22.08±1.68	13.26±1.69	24.91±2.56
5	31.14±1.97	17.85±1.42	33.36±3.01
6	41.86±2.24	23.46±2.44	42.40±2.48
7	54.70±0.99	30.88±2.98	51.95±1.44
8	68.48±3.23	38.45±2.03	63.06±2.23
9	82.07±2.74	45.58±2.77	73.06±2.43
24	99.72±2.45	57.98±2.65	86.42±1.21

LOD and LOQ

The LOD of 6-GIN, *Z*-GGS and *E*-GGS was found to be 0.260 µg mL^{-1}, 0.451 µg mL^{-1} and 0.131 µg mL^{-1}, respectively. The LOQ of 6-GIN, *Z*-GGS and *E*-GGS was 0.789 µg mL^{-1}, 1.367 µg mL^{-1} and 0.397 µg mL^{-1}, respectively.

Robustness

The robustness study revealed that the developed method was reliable and minor variations in the chromatographic conditions did not impact the peak area. The results of robustness studies is highlighted in Table VI.

Assay of actives

The content of actives in the nanoemulgel was found to be 105.28 ± 1.27%, 103.50 ± 1.23% and 94.32 ± 1.30%for 6-GIN, *E*-GGS and *Z*-GGS respectively.

In vitro diffusion study

In vitro diffusion study of nanoemulsion loaded gel was performed in phosphate buffer pH 7.4: ethanol (1:1)

Table VIII: Stability study of nanoemulgel

Active constituent	Day 0	5°C±3°C	25°C±2°C/60% ± 5%	40°C±2°C/75%± 5% RH
6-GIN (%)	105.28±0.47	105.33±0.63	105.12±1.54	104.54±0.39
E-GGS (%)	103.50±1.54	102.87±1.42	103.37±0.59	102.36±2.18
Z-GGS (%)	99.32±0.73	99.06±1.39	99.08±1.49	98.36±0.90

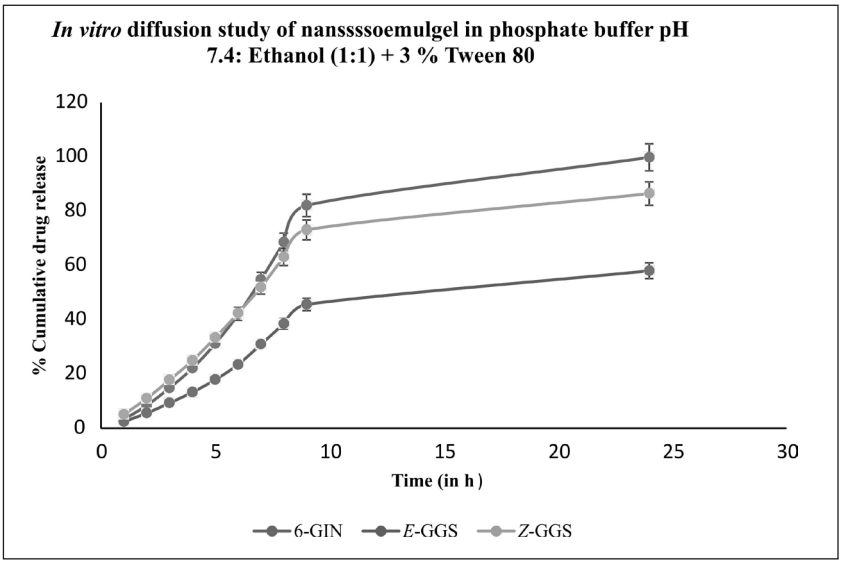

Fig. 4: *In vitro* drug release of 6-GIN, *E*-GGS and *Z*-GGS

+ 3% Tween® 80 in Franz diffusion cell using nylon membrane (0.45µm). At 24th h, around 99.72±2.45%, 57.98±2.65 % and 86.42±1.21% of 6-GIN, *E*-GGS and *Z*-GGS, respectively were released from the gel matrix (shown in Table VII and Fig. 4). However, in case of nanoemulsion, the release was faster and at 8th h, around 80% of actives were released.

Stability studies

Stability studies indicated that the developed formulation was found to be stable at all the stability conditions. Results illustrated in Table VIII indicate that there was no significant change ($p > 0.05$) in the content of 6-GIN, *E*-GGS and *Z*-GGS.

CONCLUSION

A RP-HPLC method was successfully developed for the quantitative estimation of 6-Gingerol, *E*-guggulsterone and *Z*-guggulsterone in fixed dose combination nanoemulgel. The developed method was able to quantify 6-GIN, *Z*-GGS and *E*-GGS accurately in this formulation. The developed method was reproducible and robust. The results of validation study were within the specified limits as per the guidelines. The developed method was found to be reproducible and accurate. Further, it also fulfilled linearity, precision, specificity, and suitability parameters, indicating that the method is reliable for quantification. This method can be easily adopted for analysis and quality control of 6-GIN, *Z*-GGS and *E*-GGS in fixed dose combination dosage form.

ACKNOWLEDGEMENT

We thank Rajiv Gandhi Science & Technology Commission (RGSTC) for providing funding for this project (RGSTC/File-2017/DPP-175/CR-30).

REFERENCES

1. Yin H., Zou L., Sheng Y., Bai X., Liu Q. and Yan B.: Rapid HPLC analytical method development for Herbal medicine formulae based on retention rules acquired from the constituting herbs. **Anal. Sci.,** 2018, 34(2), 207-214.
2. Saxena A., Raychaudhuri S. K. and Raychaudhuri S. P.: Rheumatoid Arthritis: Disease Pathophysiology. Inflammation, **Adv. Age Nutr. Res. Clin. Interv**., Published online 2013, 215-229.
3. Venuturupalli S.: Immune Mechanisms and Novel Targets in Rheumatoid Arthritis. **Immunol. Allergy Clin. North Am.,** 2017, 37(2), 301-313.
4. Aryaeian N., Shahram F., Mahmoudi M., Tavakoli H., Yousefi B. and Arablou T.: The effect of ginger supplementation on some immunity and inflammation intermediate genes expression in patients with active Rheumatoid Arthritis. **Gene,** 2019, 698(November 2018), 179-185.
5. Jiang T. A.: Health benefits of culinary herbs and spices.: **J AOAC Int.,** 2019, 102(2), 395-411.
6. Shahrajabian M. H., Sun W. and Cheng Q.: Clinical aspects and health benefits of ginger (*Zingiber officinale*) in both traditional Chinese medicine and modern industry.: **Acta Agric. Scand. Sect B Soil Plant Sci.,** 2019, 69(6), 546-556.
7. Van Breemen R. B., Tao Y. and Li W.: Cyclooxygenase-2 inhibitors in ginger *(Zingiber officinale).* **Fitoterapia,** 2011, 82(1), 38-43.
8. Manjula N., Gayathri B., Vinaykumar K. S., Shankernarayanan N. P., Vishwakarma R. A. and Balakrishnan A.: Inhibition of MAP kinases by crude extract and pure compound isolated from *Commiphora mukul* leads to down regulation of TNF-α, IL-1β and IL-2. **Int. Immunopharmacol.,** 2006, 6(2),122-132.
9. Bhat M. H., Fayaz M., Kumar A. and Jain A. K.: Chemistry and pharmacology of guggulsterone: An active principle of guggul plant. **Plant Hum. Heal Pharmacol. Ther. Uses,** 2019, 3, 301-319.
10. Kunnumakkara A. B., Banik K., Bordoloi D., Harsha C., Sailo B. L., Padmavathi G., Roy N. K., Gupta S. C. and Aggarwal B. B.: Googling the guggul *(Commiphora* and *Boswellia)* for prevention of chronic diseases. **Front Pharmacol.,** 2018, 9, 1-19.
11. Kamal Y. K. T. K., Singh M., Ahmad S., Alam P. and Salam S.: Stability-indicating RP-HPLC method for the determination of 6-Gingerol in polyherbal formulations. **J. Anal. Sci. Technol.,** 2015, 6(1), 1-7.
12. Weon J. B., Yang H. J., Lee B., Ma J. Y. and Ma C. J.: Simultaneous quantification of eleven chemical components in traditional herbal medicinal formula socheongryongtang by HPLC-DAD and LC-MS. **J. Liq. Chromatogr. Relat. Technol.,** 2012, 35(16), 2243-2254.
13. Bhatta R. S., Kumar D., Chhonker Y. S. and Jain G. K.: Simultaneous estimation of *E* - and *Z* - isomers of guggulsterone in rabbit plasma using liquid chromatography tandem mass spectrometry and its application to pharmacokinetic study. **Biomed. Chromatogr.,** 2011, 25(9), 1054-1060.
14. He X. G., Bernart M. W., Lian L. Z. and Lin L. Z.: High-performance liquid chromatography-electrospray mass spectrometric analysis of pungent constituents of ginger. **J. Chromatogr. A.,** 1998, 796(2), 327-334.
15. Gautam A., Paudel Y. N., Abidin S. A. Z. and Bhandari U.: Guggulsterone, a farnesoid X receptor antagonist lowers plasma trimethylamine-N-oxide levels: An evidence from *in vitro* and *in vivo* studies. **Hum. Exp. Toxicol.,** 2019, 38(3), 356-370.
16. Haque I., Kumar M. and Mukhopadhyay K.: A rapid and simple UPLC-MS-MS based simultaneous determination of the medicinally important *E*- and *Z*-guggulsterone from oleogum-resins of naturally occurring *Commiphora wightii* plants. **Chromatographia,** 2009, 70(11-12), 1613-1619.
17. Gokhale J. P., Mahajan H. S. and Surana S. S.: Quercetin loaded nanoemulsion-based gel for rheumatoid arthritis: *In vivo* and *in vitro* studies. **Biomed. Pharmacother.,** 2019, 112(July 2018), 108622.