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FORMULATION AND CHARACTERIZATION OF EZETIMIBE NANOPARTICLES FOR HYPERLIPIDEMIA TREATMENT

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The aim of the work. *Ezetimibe (EZ) is categorized as a Biopharmaceutics Classification System class II (BCS II) agent that possesses poor solubility and is highly permeable. This work aimed to incorporate EZ into nanoparticles (NPs) to accelerate release and to enhance its bioavailability.*

Materials and methods. *A stability-indicating method for the computation of EZ was developed to compute EZ in NPs in fresh and stored samples. Nine formulations of EZ-NPs were developed by solvent evaporation using HPMC 6 cps, sodium alginate, and Tween 80 in various proportions. A HPLC method was designed to estimate EZ in NPs. EZ-NPs were tested chemically and characterized.*

Results*. The developed method was fully validated, and EZ NPs comprising HPMC and tween 80 had zeta potential (ZP) ranging from –21.6 mV to –30.1 mV, higher than other formulae, and their release was enhanced. The formulation (HP4) had an elevated ZP (–30.1 mV) and released about 91% of EZ within 20 min. HP4 was chosen for stability assessment and proved to be stable.*

Conclusion*. The formulation (HP4), including 0.1:0.5:0.4 ratios of the EZ: HPMC: sodium alginate, was the optimized EZ-NPs formulation. Microencapsulation of EZ with HPMC: sodium alginate in a 0.1:0.5:0.4 ratio could enhance the release and achieve stability*

Keywords*: Ezetimibe, HPLC, sodium alginate, nanoparticles*

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1. Introduction

Hyperlipidemia is a state characterized by various genetic and acquired disorders that result in elevated lipid levels in the human body [1]. Regarding the World Heart Report 2023, 3.8 million deaths in 2021 were attributed to elevated cholesterol levels. Hypercholesterolemia management is essential to decrease cardiovascular diseases. EZ is widely utilized to diminish elevated cholesterol levels [2].

EZ is categorized as a Biopharmaceutics Classification System class II (BCS II) agent (poorly soluble and highly permeable. EZ is a novel drug employed for the management of hyperlipidemia. EZ has a low-dissolution profile, which decreases its bioavailability [3].

Hence, low solubility is a common barrier in formulation due to absorption reduction, causing unexpected medicinal levels and the threat of unfavorable responses [4]. The challenge of enhancing solubilization and managing biological variability has stimulated creative solutions, with nanotechnology occurring as a specifically favorable procedure. Nanotechnology provides solubility enhancement and absorption [5].

Solubility is a crucial parameter to execute the preferred concentration of a drug at the site of action. Many techniques can be employed to enhance solubilization and enhance their bioavailability, including chemical modification, pH adjustment, solid dispersion, complexation, and micronization [6].

In the current research, we formulated ten EZ-NPs by solvent evaporation using HPMC and sodium alginate in order to achieve rapid release. The parameters were assessed, NPs were characterized to choose the optimal formula, and then further subjected to stability assessment.

2. Research planning (methodology)

The crucial sections are:

1. The creation of HPLC for EZ.
2. Selection of excipients and formulation
3. Preparation of EZ-NPs
4. Assessment of EZ-NPs formulated
5. The optimized formula was chosen

3. Materials and methods

3. 1. Materials

EZ (HPLC, 99.8%), HPMC 6 cps, sodium alginate and tween 80 were purchased from Sigma-Aldrich, USA. Acetonitrile (AC) for HPLC; Agilent, USA. Potassium dihydrogen phosphate, sodium hydroxide pellets, hydrochloric acid, Merck, Germany.

Method development.

The procedure incorporated an Agilent HPLC and a C18 (5 µm, 25 cm × 4.6 mm) column. The mobile phase (MP) comprised 0.1 M potassium dihydrogen phosphate (pH 3) and AC in the proportion of 40:60. EZ was measured at 206 nm, and 20 ul was injected. The validation was executed per the ICH Harmonization Guideline [7, 8].

Suitability.

5 injections of a 100% standard solution were employed to assess its suitability. The parameters, namely,

plates, tailing, time, and peak areas, were acquired and ought to fulfill the limits [9].

Linearity.

Linearity in the range of 7–20 μg/mL and the standard curve were conducted to determine the slope, and the coefficient (R^2) were computed, and the area was plotted against EZ-concentrations.

Accuracy.

Accuracy implies the resemblance between expected and real values. The adequate recovery should be within the range of 90–100%, and spiking was utilized as a further quantity of EZ

Precision.

Repeatability was evaluated employing six estimations of the test. Two analysts estimate six concentrations to emphasize intermediate accuracy, and %RSD was estimated.

Precision is the extent of agreement between respective tests. The intraday precision was specified by estimating six replicates on the same day. To confine the interday precision, six replicates of EZ strengths were scrutinized for 72 h

Specificity.

Specificity is desired to demonstrate the ability to differentiate between EZ peak and other compounds ensuring no peaks overlap.

Limit of detection (*LOD*) *and limit of quantitation* (*LOQ*).

LOD of EZ that can be detected but not quantified, and LOQ of EZ that can be estimated specifically enough. LOD = 3.3 × SD/S and LOQ = 10 × SD/S were operated, where SD = standard deviation and S = slope.

Robustness.

Robustness is the capability to remain unchanged by slight changes and to reach dependability. It was assessed by analyzing the influence of changes, including wavelength and flow rate.

Forced degradation.

Alkaline hydrolysis was performed by 1 mL of 1M NaOH, boiling for 20 min, cooling, neutralizing, and completing with MP. Acid hydrolysis was performed by 2 mL of 1 M HCl, boiling for 10 min, cooling, neutralizing, and diluting with MP. Oxidative hydrolysis was conducted by 2 mL of hydrogen peroxide, boiling for 10 min, cooling, and completing with MP.

3. 2. Formulation of NPs

Polymers like sodium alginate and hydroxypropyl methylcellulose (HPMC) are utilized in NPs formulations due to their excellent drug delivery characteristics. Sodium alginate is a biocompatible natural polymer which can form NPs via ionic gelation and encapsulates drugs, protects them from degradation and achieves modified release. It also possesses muco-adhesive characteristics that enhance drug bioavailability [10]. HPMC, on the other hand, can form NPs thanks to its ability to form water-soluble films and prevent particle agglomeration. Its swelling and gelling characteristics also contribute to modulating drug release. Thus, a combination of alginate and HPMC can produce stable NPs with enhanced release characteristics [11]. Tween has been utilized as a stabilizer [12].

Nanoparticles preparation.

EZ (0.1 g) was solubilized in 25 mL of acetone by sonication. Polymers were dissolved in 100 mL of water, comprising Tween 80, and 50 mL of liquid paraffin was added. The suspension was homogenized at 25000 rpm, moved to a magnetic stirrer, and agitated till acetone completely evaporated, producing an EZ-NPs suspension. The EZ-NPs were dried and gathered (Table 1).

Table 1

Formulations of EZ

Composition per g	Formulation								
	HP1	HP2	HP3	HP4	HP5	HP6	HP7	HP8	HP9
HPMC 6 cps	0.5	0.75	1	0.5	0.75	1	–	–	–
Sodium alginate	–	–	–	0.4	0.8	1	1.2	1	1.5
Tween 80	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Acetone	25	25	25	25	25	25	25	25	25
Water	25	25	25	25	25	25	25	25	25

3. 3. Characterization of NPs

Particle size (*PS*) *and ZP investigation.*

PS, the polydispersity index (PDI), and ZP of the EZ-NPs were estimated utilized a Malvern Zetasizer, UK, in triplicate [13].

EZ-content and entrapment efficiency (*EE*).

to compute EZ-percent and EE. NPs (comprised 10 mg EZ) were dispersed in 5 mL AC and centrifuged at 30000 rpm for 15 min to isolate the supernatant, and the concentrated liquid was filtered. The EZ percent was computed by utilizing the formulae below [14, 15]:

$$\text{EZ content} = \frac{\text{Actual quantity of EZ loaded} \times 100}{\text{Weight of NPs}};$$

$$\%EE = \frac{\text{Weight of EZ loaded} \times 100}{\text{Weight of EZ initially taken}}.$$

In vitro release.

Employing a dialysis membrane, 10 mg of EZ in NPs were dissolved. Therefore, the bags were propped up and placed in the baskets of the USP tester (Erweka GmbH, Germany), apparatus type I, at 75 revolutions per minute in 250 milliliters of 0.45% SLS in 0.05 M Acetate Buffer, pH 4.5 [16]. After taking aliquots at intervals and then replacing them with new medium in order to maintain sink conditions, we estimated the percentage of EZ that had been released from the nanoparticles.

Kinetics of the EZ release.

To convey the release that best correlates with R^2 matching EZ release, the release data were fitted to release mechanisms [17] by employing the equations below:

1. Zero order

$$C = C_o - K_o t.$$

2. First-order

$\log C = \log C_o - Kt / 2.303$.

3. Higuchi diffusion

$Q = kt^{½}$,

where C_o – initial concentration; C – remaining concentration at time t; t – time of release; K_o – zero order rate constant; K – first order rate constant; Q – amount released/unit area.

3. 4. Stability

The selected EZ-NPs were stored at 30°C /65 RH for 6 months. The samples were taken at interval 0, 3, and 6 months.

3. 5. Statistical analysis

Data are demonstrated as mean ± standard deviation (SD). GraphPad Prism (version 10.6.1) was employed.

4. Results

A HPLC method was designed to assess EZ in fresh and stored samples, comprising validation parameters per ICH and USP recommendations [18]. the appropriateness was estimated; The results fulfilled the criteria as demonstrated in Table 2. The HPLC EZ-chromatogram is depicted in Fig. 1, 2. R^2 was 0.9998. The linear equation was $Y = 162.53x + 7.511$, where Y illustrates the area and X is the EZ-concentration illustrated in Table 2.

The appropriateness was established by scrutinizing a placebo and a standard solution; the absence of peaks appeared around EZ-time.

The R^2 was 0.9998 within the limits, and the intercept was 7.511 and RSD was 0.29. Furthermore, the method was verified to be precise, as the estimates of the same sample were close and RSD was < 1 or complied with the limit of <2%. LOD and LOQ for EZ were 0.31 µg/ml and 0.94 µg/ml, respectively. After a placebo injection, no peaks occurred to verify specificity. EZ remains intact in acidic and oxidative conditions, but the alkaline degradation was severe. Likewise, the method was revealed to be robust, as the RSD was <0.25% after a tiny change, as demonstrated in Table 2.

Table 2

Validation parameters

Acceptance criteria		Results
Linearity	$R^2 \geq 0.98$	0.9998
	Slope	162.53
	Intercept	7.511
	Regression equation	$y = 162.53x + 7.511$
Accuracy	Mean % recovery	99.8%
	100 ± 5%	0.29%
	RSD ≤ 2%	
Precision	Repeatability (RSD % ≤ 5%)	0.12%
	Intermediate (RSD % ≤ 10%)	0.43%
Sensitivity	LOD	0.31 µg /ml
	LOQ	0.94 µg /ml
Conditions	Acidic	99.7 ± 0.24%
	alkaline	35.76 ± 0.35%
	oxidative	99.8 ± 0.12%
Robustness RSD	1.2 mL/min	0.17%
	0.8 mL/min	0.24%
	pH 2.8	0.21%
	pH 3.2	0.23%

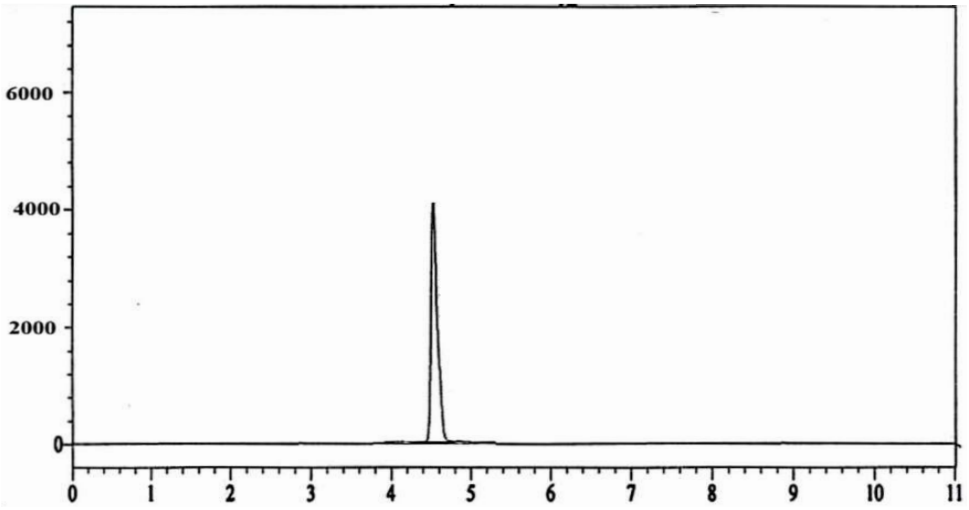

Fig. 1. The chromatogram of EZ

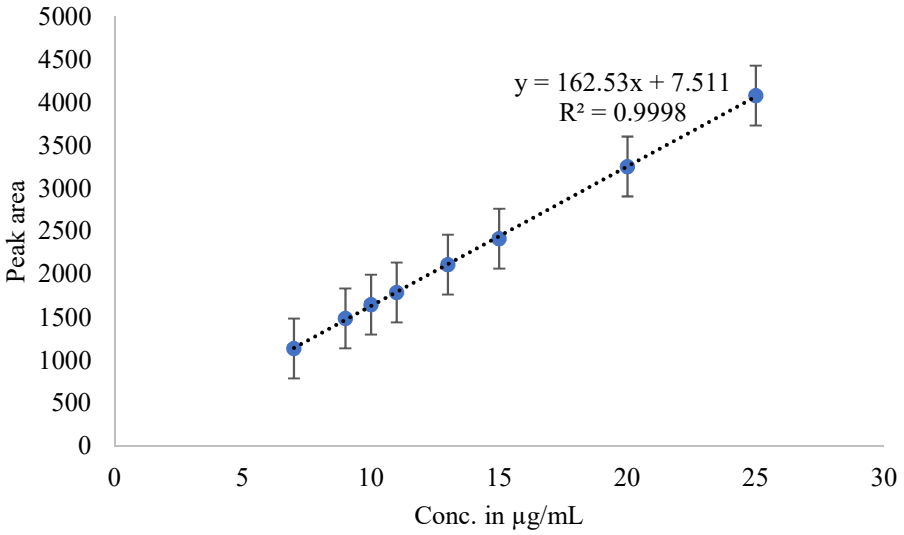

Fig. 2. The standard curve of EZ

Table 3 demonstrated that the PS of EZ-HPMC-NPs ranged from 411 to 498 nm, and their average ZP was in the range –22.7 to – 30.1 mV, while the EZ-sodium alginate-NPs PS ranged from 299 to 367 nm, and their ZP was in the range –25.1 to –26.9mV. EZ-HPMC-sodium alginate-NPs ZP ranged 28.6 to 30.1 mV. Furthermore, the HP4 nanoparticles prepared by HPMC and sodium alginate (0.1:0.5:0.4) had the highest ZP (–30.1 mV), and a PS of 388.3 nm (Fig. 3).

The EZ content of HP1 to HP9 NPs ranged from 63.45% to 89.37%, and the average EE ranged from 72.55% to 86.65%. Furthermore, the best formulation comprised sodium alginate and HPMC, as shown in Table 3.

Fig. 4 demonstrates that the release decreased with the increase of HPMC. On the other hand. The release of EZ from HPMC-NPs was less than 58% after 20 min, while NPs-sodium alginate released ≥ 70% after 20 min. The EZ-NPs comprised of HPMC 6cps with sodium alginate demonstrated high release, especially HP4 which released 91% after 20 min.

Table 3

Characterization of EZ-NPs

Formula	Mean drug content (%)	Mean Efficiency entrapment (%)	Mean ZP (mV)	Mean particle size (µm)	Mean PDI
HP1	64.26	72.55	–22.7	411	0.34
HP2	65.23	73.34	–23.9	439	0.47
HP3	63.45	72.81	–24.1	498	0.62
HP4	89.11	86.65	–30.1	456	0.36
HP5	86.25	85.11	–28.6	395	0.39
HP6	89.37	82.12	–29.1	423	0.43
HP7	76.21	76.65	–26.9	367	0.40
HP8	78.27	79.11	–25.1	348	0.37
HP9	81.38	81.12	–24.8	299	0.46

4. 1. Kinetics of the release

The release of each formulation was assessed according to the equations that best correlates with the R^2 matching the profile of EZ release and was demonstrated in Table 4 as the release of the best formula follows first order.

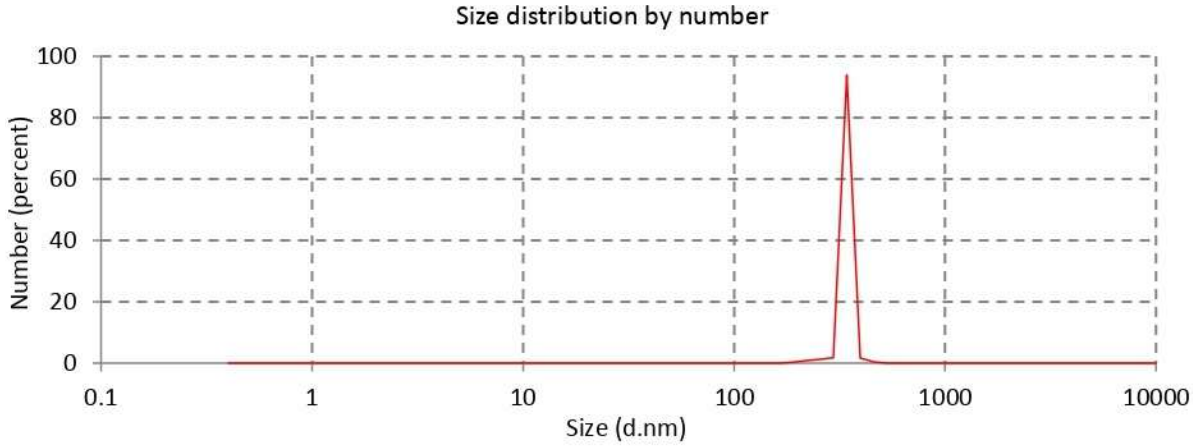

Fig. 3. The size distribution of the HP4-NPs

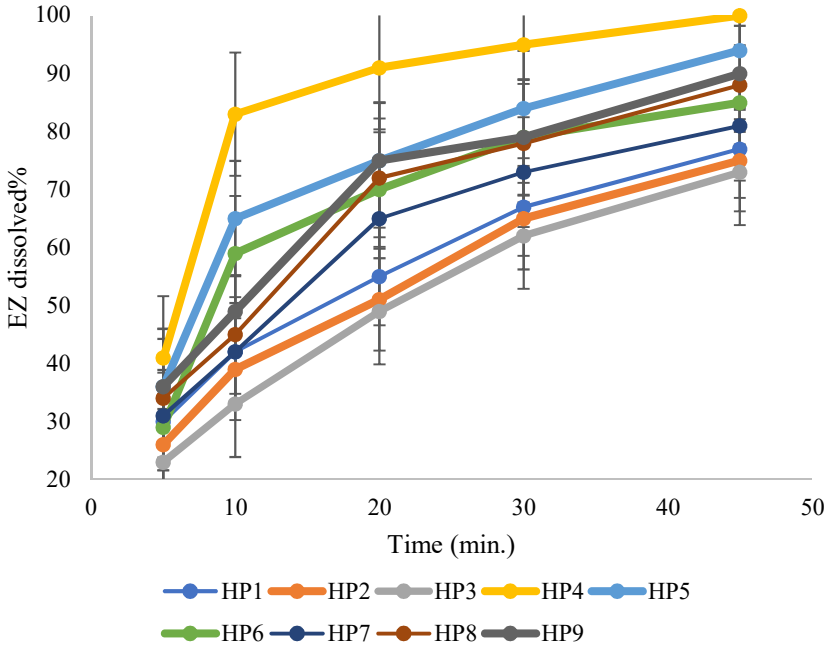

Fig. 4. Dissolution of EZ from NPs formulations

Table 4

Kinetics of EZ-NPs release

Formula	Zero order R^2	First order R^2	Higuchi diffusion R^2
HP1	0.987	0.963	0.754
HP2	0.910	0.943	0.863
HP3	0. 963	0.968	0.971
HP4	0.919	0.986	0.853
HP5	0.920	0.961	0.911
HP6	0.953	0.923	0.963
HP7	0.913	0.881	0.905
HP8	0.891	0.931	0.871
HP9	0.982	0.991	0.978

Note: R^2 – correlation coefficient.

The selected was subjected to storage conditions and the samples were assessed for EZ at the specified intervals as in Table 5.

Table 5

Assay of EZ after storage

Formula	0 time	3 months	6 months
HP4	101.2	100.9	100.5

5. Discussion

A HPLC method was designed to assess EZ in both fresh and stored samples, incorporating validation parameters as per ICH and USP guidelines [18]. Prior to investigating the true samples, the appropriateness was computed; the tailing factor (T) should be < 2, the capacity factor (k') should be > 2, and the plate count should be ≥ 2000 [19]. Therefore, the results fulfilled the criteria as displayed in the results

Appropriateness was achieved by scrutinizing a placebo and a standard solution; the absence of peaks around EZ-time demonstrated that the method is specific.

The R^2 was 0.9998 within the limits (not less than 0.99), and the intercept was 7.511. The method proved accurate, as it recovered 99.8%, and RSD was 0.29. Furthermore, the method was verified to be precise, as the repeated estimates of the same sample showed minimal variation, demonstrated by an RSD of less than 1% and compliance with the accepted limit of less than 2% [20, 21]. LOD and LOQ for EZ were 0.31 µg/ml and 0.94 µg/ml, respectively. After a placebo injection, no peaks occurred to verify Specificity. EZ remained intact in acidic and oxidative conditions, but alkaline degradation was severe. Likewise, the method was exhibited to be robust, as the RSD was <0.25% after a minor change.

The study provided formulations of EZ-NPs containing suitable polymers, namely, HPMC 6 cps, sodium alginate. The EZ-HPMC-NPs were characterized by range of size, from 411 to 498 nm, and ZP was in the range –22.7 to –30.1 mV, while the EZ-sodium alginate-NPs PS ranged from 299 to 367 nm, and their ZP ranged from 25.1 to –26.9 mV. EZ-HPMC-sodium alginate- NPs ZP ranged 28.6 to 30.1 mV. Furthermore, the HP4 nanoparticles prepared using HPMC and sodium alginate (0.1:0.5:0.4) exhibited the highest ZP (–30.1 mV), displaying a PS of 388.3 nm and optimal stabilization and freedom from aggregation over time. PDI was uniform in all NPs prepared, except in HP3 with HPMC 0.62. However, the PDI of the remaining NPs prepared ranged from 0.37 to 0.48, due to the uniformity of PS [22]. Fig. 3 reveals the size distribution of formula HP4, with a mean diameter of 456 nm, confirming that its size falls within the nanorange.

The EZ content of HP1 to HP9 NPs ranged from 63.45% to 89.37%, and the average EE ranged from 72.55% to 86.65%. Furthermore, the best formulation comprised sodium alginate and HPMC. The release decreased with increasing of HPMC concentration. On the other hand. The release of EZ from HPMC-NPs was less than 58% after 20min, while NPs-sodium alginate released ≥ 70% after 20 min. The EZ-NPs comprised of HPMC 6 cps with sodium alginate demonstrated high release, as HP4, which released 91% after 20 min. The HP4 was chosen for stability testing to maintain the content of EZ as the degradation was less than 1%.

Most attempts to encapsulate EZ are to sustain its release, such as Elkhayat et al. developed EZ-loaded nanostructured lipid carriers (EZ-NLCs) to enhance their dissolution, employing croscarmellose sodium and mannitol. The optimized EZ-NLC tablet exhibited rapid disintegration time, and 98% of the EZ released within 24 h [23].

Moreover, Tulain et al formulated *Linum usitatissimum* mucilage-based NPs, to encapsulate EZ by solvent evaporation and nanoprecipitation techniques. Developed nanoparticles were characterized. The EZ release demonstrated 80% within 24 h [24].

In the current work, EZ-NPs were formulated as the first attempt to microencapsulate EZ using sodium alginate and HPMC 6cps. These were converted into rapid-release NPs with enhanced characteristics, including high EZ content and outstanding EE. Among the nine NPs formulations, HP4 showed 91% release of EZ after 20 minutes. Therefore, HP4 was identified as the optimized formulation, possessing the most satisfactory characteristics. Stability assessment further proved its high stability. The current results are comparable to those of Ali et al. (2015), who prepared EZ-NPs with various polymers, including polyvinyl pyrrolidone (PVPK-30), polyvinyl alcohol (PVA), HPMC E5, and poloxamer. The best formula had observed an increased dissolution rate and a comparable percentage of dissolved material [6] and Torrado-Salmerón et al., 2020 formulated EZ-NPs employing Kolliphor® RH40 as a surfactant and croscarmellose, which significantly enhanced the dissolution as it dissolves about 90% of EZ which is comparable to the current results [3]. However, in the current study, different polymers were used, and the chosen formula was tested and found to be stable.

Practical relevance. HPLC was developed for assessing EZ in nanoparticles and their stability. Physicochemical characteristics and stability of EZ-NPs were assessed. The study can be beneficial for assessing EZ stability in other dosage forms and for formulating EZ in

combination with other compounds of similar chemical structure.

Research limitations. The developed method may not be able to assess EZ in combination with other antihyperlipidemic.

Prospects for further research. Additional investigations include EZ-prepared nanoparticles' bioavailability testing and studies on the combination of EZ with other antihyperlipidemic agents in nanoparticles.

6. Conclusion

The study developed a robust RP-HPLC method to assess EZ, which proved accurate, precise, and stability-indicating. EZ nanoparticles were efficiently prepared employing HPMC and sodium alginate, with the HP4 formulation showing optimal stability and rapid drug release. Polymer composition strongly influenced EZ release, with sodium alginate–based nanoparticles enabling the fastest release. Overall, the validated HPLC method and optimized nanoparticles offer a promising approach to enhance EZ dissolution and performance.

Conflict of interest

The authors declare that they have no conflict of interest related to this research, whether financial, personal, authorship or otherwise, that may influence the research and its results demonstrated in this manuscript.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm that they did not employ artificial intelligence technologies in creating the submitted work.

Authors' contributions

Asmaa Mohamed: design methodology, analysis, and supervision, **Olla Maan:** design methodology, analysis, writing, **Firas Rahi**: design methodology, analysis, writing, **Doaa Elkashif:** analysis, writing.

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