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Research article

Greenness assessment of micellar spectrofluorometric approach for determination of Elagolix: application to dosage form, content uniformity and human plasma

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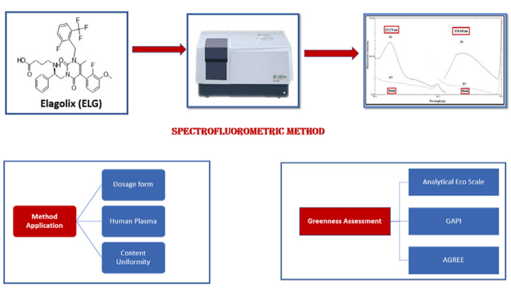
GRAPHICAL ABSTRACT



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ABSTRACT

Ecological, sensitive, fast and economic approaches are the main aspects in quality control of pharmaceutical products. Elagolix (ELG) is an orally non-peptidic GnRH antagonist, recently approved drug by Food and drug administration in 2018 for treatment of pain associated with endometriosis. A green, and sensitive method was developed and validated for determination of ELG based on micellar spectrofluorometric approach. Many factors were studied to enhance the fluorescence intensity of ELG and the highest sensitivity was obtained upon using 1% Sodium dodecyl sulphate (SDS) at 438 nm after excitation at 270 nm. A linear relationship was obtained over a range of 50–1000 ng mL^{-1} between ELG concentration and corresponding fluorescence intensity. The developed method was validated according to ICH guidelines and successfully applied for testing the content uniformity and determination of ELG in pharmaceutical dosage forms with percentage recovery 99.31 $\pm$ 1.98. Furthermore, the capability of the method due to its high sensitivity to determine ELG in human plasma with percentage recoveries in a range of 98.54–100.46. The greenness of the method was investigated using three different approaches; Analytical Procedure Index (GAPI), Analytical Eco-Scale and Analytical Greenness Metric (AGREE).

1. Introduction

Endometriosis is one of the common chronic gynaecological disorder which afflicts women of reproductive age and its occurrence is rare in postmenauposal women [1]. This disorder with its related symptoms significantly affects the psychological behaviour of woman and her quality of life [2, 3, 4]. Endometriosis creates when tissues found within the uterus starts to develop outside of it. Such growth is referred to as injuries and

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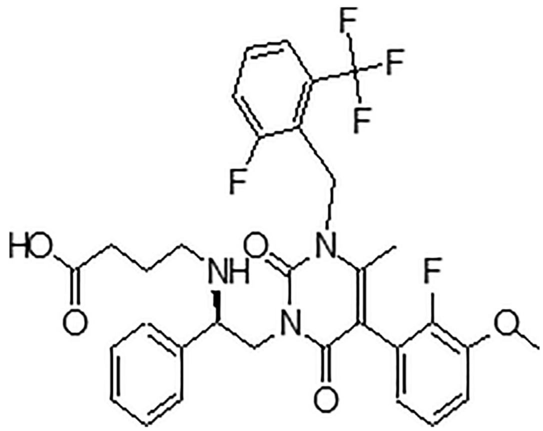

Figure 1. Chemical structure of ELG.

leads to various symptoms like pelvic pain and infertility [5, 6]. The development of these injuries is subordinate upon the estrogen hormone. In the management of pain associated with endometriosis, the first line therapy is pain reliever (non-steroidal anti-inflammatory drugs) [7].

Elagolix (ELG) 4-[[(1R)-2-[5-(2-Fluoro-3-methoxyphenyl) -3-[[2-fluoro -6- (trifluoromethyl) phenyl] methyl] -4- methyl -2, 6-dioxopyrimidin-1-yl]-1-phenylethyl]amino] butanoic acid (Figure 1), is an oral, first and short acting gonadotropin-releasing hormone(GnRH) antagonist drug which approved by FDA in 2018 for treatment of pain associated with endometriosis [8]. The cited drug reduces the pelvic pain by inhibiting GnRH signaling through competitively binding to GnRH receptors [9, 10].

The literature survey reveals that one reported analytical method is developed for separation of ELG isomers [11]. No analytical method has been reported for determination of ELG neither in pharmaceutical products nor in biological fluids.

Over the past two decades, one of the key goals of the analytical chemistry has been to implement green analytical procedures. Green Chemistry aims to reduce or eliminate the use or generation of hazardous substances. Many aspects of the analytical method development as sample size, sample preparation and extraction techniques can be modified to comply with green chemistry principles. Implementation of green chemistry can take numerous forms, ranging from re-designing experiments with eco-friendly reagents to waste management. Many assessment tools have been developed to compare the efficacy of green chemistry techniques against traditional techniques [12].

The aim of this work was to use the native fluorescence nature of ELG molecular structure to develop a spectrofluorometric method. The method was based on enhancement of fluorescence intensity by addition of sensitizing agent and form micellar environment to sequester ELG from any quenching effect. Accordingly economic and green solvents were used and factors affecting both native and enhanced fluorescence were studied to optimize measuring factors. The developed spectrofluorometric method was validated and successfully applied for the quantification of ELG in bulk powder, pharmaceutical dosage form and human plasma with high sensitivity. In addition, the greenness of the developed method was assessed using Green Analytical Procedure Index (GAPI) [13], Analytical Eco-Scale [14] and Analytical Greenness Metric (AGREE) [15].

2. Experimental

2.1. Instruments

Shimadzu spectrofluorometer (model: RF 5301 PC, Japan), equipped with 150-watt Xenon lamp was used to perform fluorescence measurements. Slit widths for monochromators were set at 10 nm and 1 cm quartz cell was used. pH measurements were performed using Jenway pHmeter 3510 (Cole-Parmer, Staffordshire, UK). Vortex Stuart® (SA8, United Kingdom) and cooling centrifuge Sigma® (3-30KS, Germany) were used.

2.2. Materials and reagents

Elagolix (99.99 %) was kindly supplied by Hekma pharmaceutical industries, Cairo, Egypt. Orlissa® tablets (1122931) were purchased from local pharmacies and each tablet contained 150 mg of elagolix. All solvents and materials used throughout the work were of analytical grade. Ethanol, methanol and acetonitrile, all of HPLC grade, were purchased from (Sigma-Aldrich, Germany). Sodium acetate, sodium dihydrogen orthophosphate, hydrochloric acid, sodium hydroxide, sodium carbonate and borax were purchased El-Nasr Pharmaceutical Chemicals Co. (Egypt). Sodium dodycyl sulphate (SDS), triton x100, carboxy methyl cellulose (CMC), tween 20, tween 80 and cetrimide were Belami fine chemicals (Mumbai, India). Human plasma was purchased from Vacsera National Blood Bank, (Giza, Egypt).

2.3. Standard solution of ELG

A stock solution of ELG was prepared in distilled water by dissolving 100 mg of the drug in a 100-mL volumetric flask to reach a concentration of 1000 μg mL^{-1}. Stock standard solution was stored for 14 days in refrigerator at 4 °C. A series of working standard solutions with concentration range between 0.5 μg.mL-1- 40 μg mL^{-1} were prepared in 25-mL volumetric flask by transferring different aliquots (12.5–1000 μL) from stock solution then diluting using the same solvent.

2.4. Preparation of studied surfactants

Preparation of stock solution of studied surfactants was carried out by dissolving 1.0 g or 1 mL of each surfactant in 100 mL distilled water in a series of 100-mL volumetric flasks. The used surfactants in this study were 1% SDS (w/v), 1% triton x100 (v/v), CMC (w/v), 1% tween 20 (v/v), 1% tween 80 (v/v) and 1% cetrimide (w/v).

2.5. Construction of the calibration curve

Aliquots of ELG working standard solution (1 mL) containing different concentrations of ELG were quantitatively transferred into a set of 10-mL volumetric flasks; followed by adding 1 mL of 1% SDS (W/V) and all the volumetric flask were completed to the mark with distilled water. The prepared solutions were diluted to 10 mL with distilled water and mixed thoroughly to reach final concentrations in the range of 50–1000 ng mL^{-1}. The solutions were measured at 438 nm after excitation at 270 nm. Blank experiments were carried out and fluorescence intensities were determined. Relative fluorescence intensity (RFI) values were plotted against corresponding concentrations to construct the calibration curve then regression equation was computed.

2.6. Analysis of ELG pharmaceutical dosage form

Fourteen Orlissa® tablets were accurately weighed, finely grinded and mixed. An accurately weighed amount of the powdered tablets corresponding to 100 mg of ELG was transferred into 100-mL volumetric flask followed by addition of 20 mL of distilled water. The solution was sonicated for 30 min then followed by filtration and dilution with distilled water to reach final concentration of 1000 μg mL^{-1}. Into 100 mL-volumetric flask, 1 mL of the filtrate was transferred and diluted to reach a concentration of 1 μg mL^{-1}. Different aliquots of the prepared solution were transferred into 10 mL-volumetric flasks followed by addition of 1 mL of 1% SDS (W/V) then diluted to the mark with distilled water. RFI of prepared solutions was measured at 438 nm after excitation

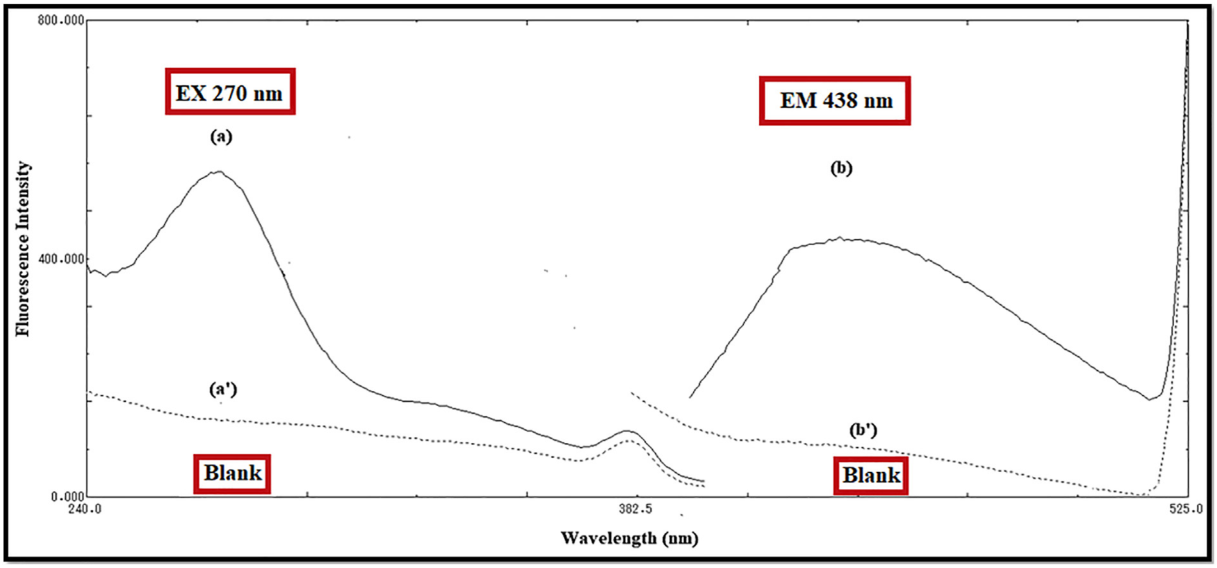

Figure 2. Excitation (a', b') and emission spectra (a,b) of ELG in SDS system.

at 270 nm and the corresponding concentrations were calculated using the computed regression equation.

2.7. Content uniformity testing

The content uniformity testing was assessed and performed through analysis of ten tablets individually according to official USP guidelines [16]. Each tablet was individually weighed, crushed, and analysed as the previously mentioned procedures in section (2.5.). The percentage recoveries were calculated and the acceptance value was calculated.

2.8. Analysis of ELG in spiked human plasma

Aliquots of human free drug plasma (750 μL) were spiked using adjustable micropipette (100–1000 μL) with1.25 mL of ELG working standard solution containing different concentrations of ELG in a set of centrifuge tubes separately. The protein precipitation was carried out for extraction method by addition of 3 mL acetonitrile in each centrifuge tube for complete precipitation of protein [17, 18]. The prepared mixtures were vortexed for 1 min then centrifuged for 15 min at 4000 rpm. From each centrifuge tube, the supernatant was removed and filtered then 1 mL of filtered supernatant was transferred into 10 mL-volumetric flask followed by addition of 1 mL 1% SDS (W/V) then addition of distilled water up to the mark to reach concentration range between 50 ng.mL^{-1}-1000 ng mL^{-1}. The fluorescence intensities were measured at 438 nm after excitation at 270 nm and the blank experiments were carried out in the same way without addition of ELG standard solution.

3. Results and discussion

ELG was selected in this work because it is a new FDA approved drug and there is no analytical method was found for its quantification in its dosage form or in biological fluids. ELG shows a native fluorescence at wavelength of 438 nm after excitation at 270 nm as presented in (Figure 2). Many factors affecting the native fluorescence of ELG were studied in this work using one factor at a time (OFAT) experiments as solvent effect, organized media effect, volume and percentage of surfactant effects and pH effect.

3.1. Effect of experimental parameters

3.1.1. Effect of diluting solvent

The effect of solvent on fluorescence intensity was studied without use of micellar organized microenvironment by using two types of solvents; protic solvents and aprotic solvents. Protic solvents are solvent that have hydrogen atom attached to electronegative atoms as oxygen or nitrogen that allow to form hydrogen bonding such as water, methanol and ethanol. While aprotic solvents don't have hydrogen atoms attached directly to electronegative atoms as acetone, acetonitrile and ethyl acetate. Distilled water was chosen as diluting solvent as shown in

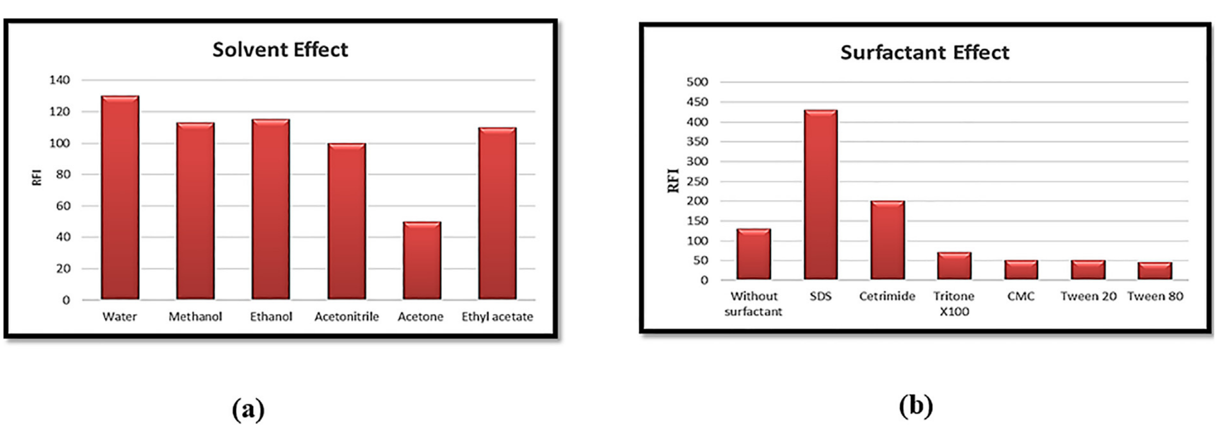

Figure 3. (a) Effect of different solvents on the fluorescence intensity of ELG. (b) Effect of different organized media on the fluorescence intensity of ELG.

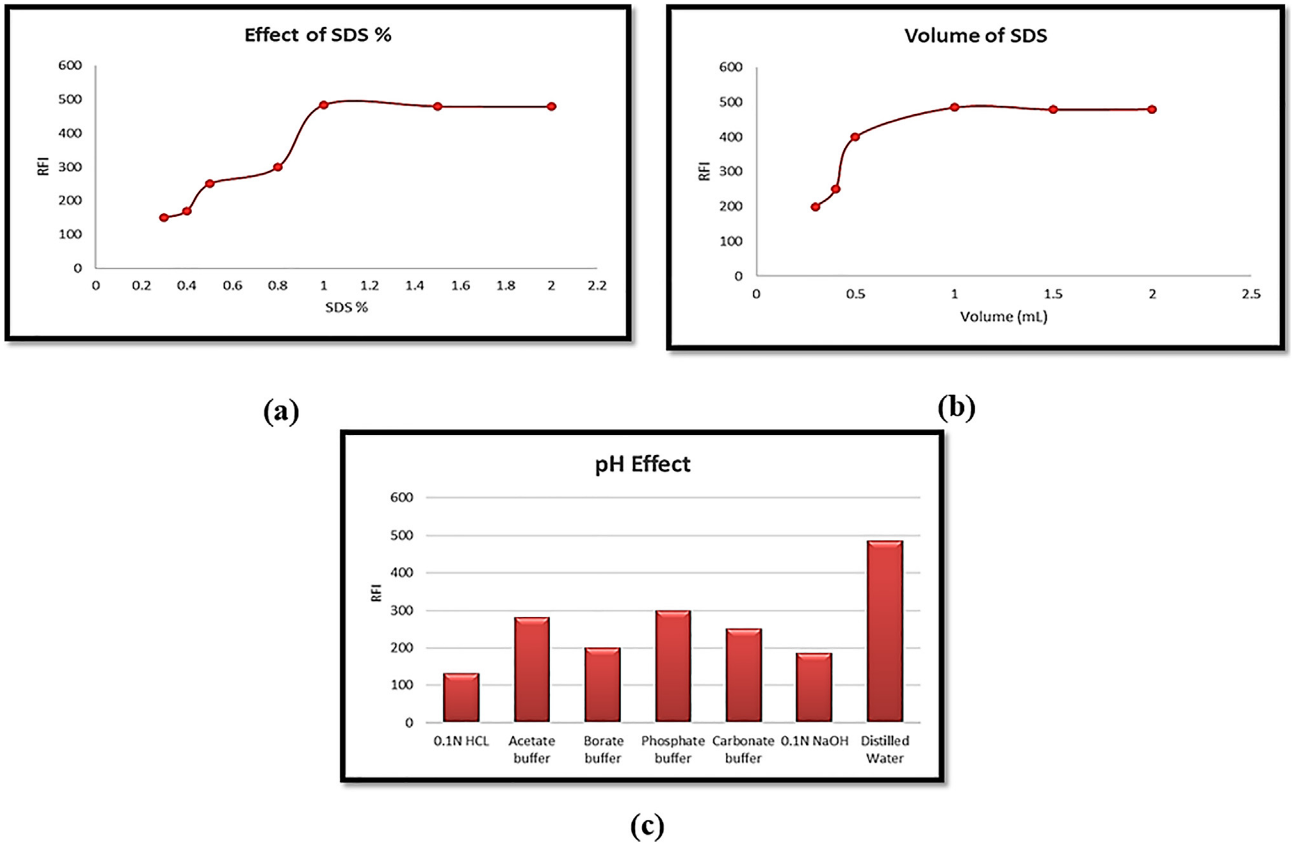

Figure 4. (a) Effect of % of SDS on the fluorescence of ELG. (b) Effect of volume of SDS on the fluorescence of ELG. (c) Effect of pH on the fluorescence of ELG in SDS system.

(Figure 3a) as distilled water gave higher fluorescence intensity and allow the method to be environmentally safe and economic.

3.1.2. Effect of organized media

Enhancement of fluorescence intensity of ELG was performed by studying different organized media including anionic surfactant (SDS), non-ionic surfactant (Tween 80, tween 20 and Triton X-100), cationic surfactant (cetrimide) and anionic polymer (CMC). The effect of each surfactant on fluorescence intensity of ELG was evaluated separately by adding 1 mL of 1% of each surfactant to ELG solution then measuring RFI. As shown in (Figure 3b), it was observed that RFI was significantly increased by four-folds when addition of anionic surfactant (SDS) compared with aqueous solution while decreased when using the other surfactants.

3.1.3. Effect of surfactant concentration

Different concentrations of SDS ranging from 0.2-2% (W/V) were examined to study the effect of concentration of SDS on RFI of ELG as this concentration above the critical micelle concentration (CMC) of SDS. The obtained results revealed that a concentration of 1 % (W/V) of SDS was optimum concentration to give the highest RFI and above this concentration there was no increase in RFI of ELG as shown in (Figure 4a).

3.1.4. Effect of surfactant volume

The effect of SDS volume on RFI of ELG was investigated by using different volume of 1% SDS (W/V) ranging from (0.1–2 mL). It was observed that 1 mL gave the highest RFI compared with the other volumes and no enhancement in fluorescence intensity was noticed while increasing the volume above 1 mL of 1% SDS as shown in (Figure 4b). Therefore, the optimum volume of 1% SDS (W/V) was 1 mL to maximize the fluorescence of ELG.

3.1.5. Effect of pH

The pH effect on RFI of ELG in 1% SDS (W/V) system was evaluated using different buffers to select the suitable pH with optimum micelle enhanced fluorescence intensity. The buffers that included in this study were: HCL (0.1N), acetate buffer (0.1M), phosphate buffer (0.1M), borate buffer (0.1M), carbonate buffer (0.1M) and NaOH (0.1M) covering pH ranges of (1–2), (3.5–4.5), (5.5–7.5), (8.5–9.5), (10–11) and (11–13) respectively. It was observed that the highest RFI was found upon using distilled waterwhile significant decreasing in RFI values in acidic and alkaline conditions as presented in (Figure 4c). Selection of distilled water revealed that this solvent was the suitable environment for micelle formation and gave the ability for SDS to enhance ELG fluorescence nature.

3.2. Method validation

The validation procedure of the developed analytical method was carried out by following the ICH guidelines [19]. The method parameters that investigated in this study were linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy.

3.2.1. Linearity

The linearity of the developed method was confirmed by preparation of solutions at five different concentrations from the working standard solution of ELG. Construction of calibration curve was carried out by plotting the RFI of ELG as a function of concentration in ng.mL^{-1}. The regression equation was calculated to be $Y_1 = 0.32x + 166.49$, and the correlation coefficient was found to be 0.9994, as presented in (Table 1), revealing satisfactory linearity in the proposed spectrofluorometric method. The linear range are evaluated by construction of residual plot, fit plot and normal probability plot to test the homoscedasticity indicating acceptable linear range as shown in S1.

Table 1. Analytical parameters of the proposed spectrofluorometric method for determination of ELG.

Parameter	Spectrofluorometric Method
λ_{ex}	270
λ_{em}	438
Linearity:	
Regression equation	$Y_1 = 0.32x + 166.49$
Range (ng.mL^{-1})	50–1000
Correlation coefficient (r)	0.9994
Slope	0.32
Intercept	166.49
S.D of slope	0.002
S.D of intercept	1.60
LOD(ng.mL^{-1})	16.50
LOQ (ng.mL^{-1})	50.0
Precision:	
Repeatability (Intraday) (% RSD)*	
QCL (200 ng mL^{-1})	1.20 %
QCM (500 ng mL^{-1})	0.48 %
QCH (800 ng mL^{-1})	0.29 %
Intermediate precision (Inter-day) (% RSD)*	
QCL (200 ng mL^{-1})	0.18 %
QCM (500 ng mL^{-1})	0.86 %
QCH (800 ng mL^{-1})	0.29 %
Accuracy: (Mean ± SD)**	
QCL (200 ng mL^{-1})	99.86 ± 0.88
QCM (500 ng mL^{-1})	98.72 ± 0.26
QCH (800 ng mL^{-1})	101.07 ± 0.16

* RSD: relative standard deviation.
** Expressed mean of three replicates.

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

The estimation of LOD and LOQ by using the equations: LOQ = 10 σ/S and LOD = 3.3 σ/S, where σ is the standard deviation of the intercept, and S is the slope of the calibration curve. LOD and LOQ were found to be 16.5 and 50.0 ng mL^{-1} respectively. The observed results as shown in (Table 1) confirm the high sensitivity of the proposed method.

3.2.3. Precision

The precision of the proposed method was investigated by preparing and analysing solutions of three different concentrations of ELG on the same day (repeatability) and the three successive days (intermediate precision). Low values were observed after calculation of relative standard deviations indicting the precision of the proposed method (Table 1). Therefore, the developed method is sufficiently precise.

3.2.4. Accuracy

The accuracy of the method was assessed by analysing three replicates of three different concentrations then calculating the percentage recoveries as shown in (Table 1). Satisfactory percentage recoveries were obtained (between 98.72% and 101.07%) after using the regression equation to calculate the concentrations. Hence, the developed spectrofluorometric method is accurate.

3.3. Applications

3.3.1. Analysis of ELG in pharmaceutical dosage form

The proposed method was successfully applied for determination of ELG in pharmaceutical dosage form, and the obtained results were found to be satisfactory with a good recovery (99.31%). Verification of the method was assessed by performing standard addition technique and the results indicating that there was no interference from excipients found as presented in (Table 2).

Table 2. Application of the proposed method for determination of ELG in pharmaceutical dosage form and application of standard addition technique.

Dosage form	Claimed (ng.mL^{-1})	% Found (Mean ± SD)	Standard addition technique	
			Pure added (ng.mL^{-1})	% Recovery** (Mean ± SD)
Orlessa ®tablets (contains150 mg elagolix)	400	99.31 ± 1.98	100	101.23 ± 0.18
			400	101.74 ± 0.50
			600	101.12 ± 0.51

** Average of three determinations.

3.3.2. Content uniformity of ELG in its pharmaceutical dosage form

The evaluation of content uniformity test for ELG was performed by applying the general procedure according to USP guidelines [16]. The content of individual dosage form was assayed then percentage recoveries were calculated using the regression equation. The acceptance value (AV) was calculated by applying the following equation: AV = |M-X\|+ ks, where M is a reference value and equal to X\ when 98.5 ≤ X\ ≤ 101.5, X\ is the mean of the individual contents expressed in percentage, K is an acceptability constant (2.4) when n = 10 and S is the standard deviation. As presented in (Table 3), the acceptance values was found to be lower than the K that indicating a desired content uniformity of ELG in its pharmaceutical dosage form.

3.3.3. Analysis of ELG in spiked human plasma

Determination of ELG in spiked human plasma was carried out with regard to its therapeutic level [20]. According to the pharmacokinetics of ELG, the peak plasma level (C max) of cited drug was found to be 574 ng mL^{-1} for 150-mg dose and 774 ng mL^{-1} for 200-mg dose. The proposed method was assessed for its applicability to quantify ELG in human plasma samples. Protein precipitation with acetonitrile was selected as extraction method of ELG in human plasma as the percentage of extraction was high (98.85%) compared to extraction with ethanol and methanol. A plasma calibration curve was constructed as shown in S2 to quantify ELG after spiking and found to be linear in a range of 50–1000 ng mL^{-1}. The regression equation was calculated ($Y_2 = 16.42\,x + 204.4$) and correlation coefficient was found to be 0.9991. The selectivity was evaluated using different lots of blank plasma. Blank plasma were prepared and analyzed and RFI were compared with another set of plasma

Table 3. Application of the proposed method for determination of content uniformity of ELG in pharmaceutical dosage form.

Tablet number	% Recovery of the content claimed*
1	99.73
2	99.13
3	98.67
4	99.4
5	98.57
6	99.83
7	100.20
8	100.03
9	99.03
10	99.01
Mean	99.36
SD	0.57
RSD	0.57
Acceptance value (AV)	1.37
Max. allowed AV (L1)	15

* The values is the mean of three determinations.

Table 4. Application of the proposed method for determination of studied drug in spiked human plasma.

Amount taken (ng.mL^{-1})	Amount found* (Mean ± SD)	% Recovery± SD	% CV**
50	49.27 ± 0.51	98.54 ± 0.91	0.93
200	99.64 ± 1.37	99.64 ± 1.37	1.37
500	496.59 ± 2.68	99.32 ± 0.54	0.54
800	803.66 ± 3.48	100.46 ± 0.43	0.43

* Average of five determinations.
** Coefficient of variation.

spiked with the analytes at lowest concentration (50 ng mL^{-1}). The assessment confirmed that there was no interference from matrix components upon analysis of ELG. Matrix effect was examined and the RFI of ELG in four different concentrations prepared in extracted plasma and was compared to those obtained from those prepared in distilled water at the same concentration. The percentage recoveries were calculated and satisfactory results were observed revealing no interference from plasma endogenous components as shown in (Table 4).

3.4. Investigation of method greenness

The proposed method has been evaluated in regards to its greenness using green analytical procedure index (GAPI) which provides information on the whole procedure starting from sampling to final determination. The GAPI pictogram is composed of 5 significant colored pentagrams which are divided into 15 sections wherein each and every section illustrate an analytical step. Three levels of colors are used in GAPI to evaluate the ecological effect; green, yellow, and red, where red indicates bad effects, while yellow and green indicate medium and low ecological effects, respectively as represented in Figure 5a. Evaluation of the proposed spectrofluorometric method through GAPI shows that *4 red sections (5, 6, 7, and 15) are observed inside the pictogram which addresses sample treatment (by macro-extraction method), solvents used (acetonitrile for plasma extraction), and waste management (No waste treatment)*, and waste management (No waste treatment), respectively. Also, Four yellow sections (9, 10, 11, and 14) were observed and address the amount of solvents and reagents (10–100 mL), toxicity of reagents (moderately toxic), safety hazards (Acetonitrile with flammability score of 3) and volume of waste (less than 10 mL), respectively. The green color of other sections represents low ecological effect of the specified analytical steps.

Table 5. The penalty points of the proposed method according to the analytical Eco-Scale.

	Value	Penalty points
Reagents		
Water	<10 mL	0
SDS	<10 mL	0
Acetonitrile	<10 mL	4
Instrument		
Spectrofluorometry	≤0.1 kWh	0
Occupational hazard	Analytical process hermetization	0
Waste		
Waste amount	1–10 mL	3
Waste Treatment	No treatment	3
Total penalty points		**∑ 10**
Analytical eco-scale total score		**100 – 10 = 90**

In the proposed method, the in-line sample collection was applied (section 1) and no preservation, transport or storage (sections 2-4) was required with no additional treatment for samples (section 8). Energy consumption was less than 0.1 kWh per sample with low occupational hazards (˂1-mL) because of using spectrofluorometer (sections 12 & 13). The analytical eco scale is a quantitative tool to evaluate the greenness of the method. The ranking of the greenness of analytical method and its parameters is identified through penalty point calculations with a final score. Analytical eco-scale has been applied to the proposed method as indicated in Table 5 and the score was found to be 90 revealing excellent greenness of the proposed method as analytical eco-scale more than 75 represents excellent green analytical method. Additionally, the greenness of the proposed method was investigated using AGREE software. This software is a calculator that is used to investigate the analytical procedure and its corresponded environmental hazards through evaluating 12 parameters of green analytical aspects and each parameter represents one of the green analytical chemistry standards. A diagram and a final score are the result of this evaluation. Figure 5b represents the twelve parameters with different colors ranging from dark green that addresses low ecological effects to red color that addresses bad ecological effects based on information illustrated by Marek Tobiszewski [15]. All the sections in the diagram are green in color, except section 7 was yellow, as the amount of waste exceeds 1 mm while section 11 was orange as acetonitrile is a toxic reagent with high flammability score. Also, the

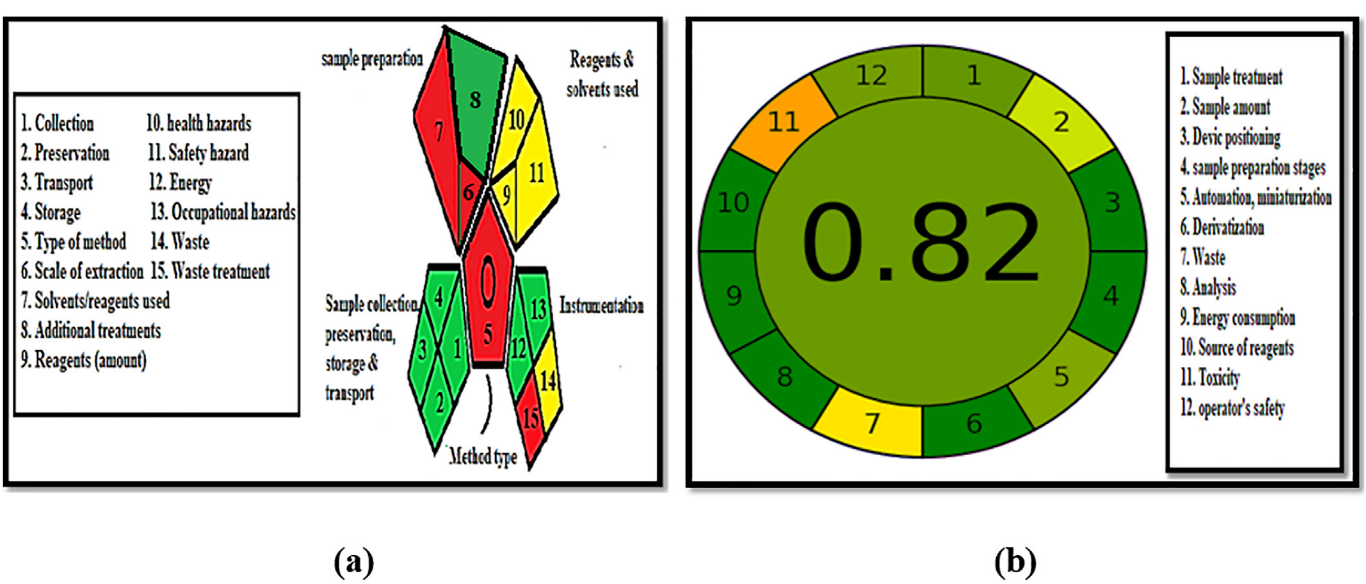

Figure 5. (a) GAPI pictogram for evaluation of the proposed method greenness. (b) Analytical greenness metric for evaluation of the proposed method greenness.

calculated score in the diagram ranging from 0 to 1 and the higher scores indicates excellent high green characteristics. In the proposed spectrofluorometric method, the score was found to be 0.82 indicating the greenness of the method.

4. Conclusion

This work presents a green micelle enhanced spectrofluorometric method for determination of elagolix. The proposed method is the first analytical method for quantification of elagolix. The native fluorescence was enhanced by 4-folds upon addition of SDS the powerful micelle forming. It was successfully applied for determination of cited drug in its marketed dosage form and testing the content uniformity; so it could be used for routine analysis in quality control laboratories for determination of elagolix qualitatively and quantitatively. Furthermore, the method was capable to quantify the studied drug in human plasma because of high sensitivity. The proposed method is considered an eco-friendly approach that will minimize the hazardous effects in laboratories.

Declarations

Author contribution statement

Rasha M. Ahmed: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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