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Research Article

Analytical Method Development and Validation for the Evaluation of Elagolix and its Impurities by RP-HPLC

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Abstract

Elagolix is used for the treatment of endometriosis. A new RP-HPLC method (Gradient mode) has been developed for the estimation of Elagolix API and its four impurities such as Elagolix impurity-1, Elagolix impurity-2, Elagolix impurity-4 and Elagolix impurity-5 using Waters Alliance system with Inertsil C8 analytical column (PDA detector). Chromatographic experiments were performed on a Waters alliance Model HPLC system, and the separation was carried out on an Inertsil and column temperature was 35ºC. A mixture of phosphate buffer solution (pH adjusted to 3.5 with ortho phosphoric acid) and Acetonitrile was used as mobile phase for the chromatographic study (Detection wavelength: 275 nm). The flow rate was 1.0 mL/min and the total run time was 40 min. Stress degradation studies were performed and the method was validated as per ICH guidelines.

Keywords: Elagolix Sodium; RP-HPLC; Impurities; Stability Indicating; Validation; ICH Guidelines

Introduction

Elagolix sodium (Figure 1) is an oral non-peptide gonadotropin-releasing hormone antagonist which was the first approved for the treatment of endometriosis [1-3]. It is chemically 4-[[(1 R)– 2-[5-(2-fluoro-3-methoxy phenyl)– 3-[[2-fluoro-6-(tri fluoro methyl) phenyl] methyl]– 4-methyl-2,6-dioxo pyrimidin-1-yl]– 1-phenyl ethyl] amino] butanoate sodium.

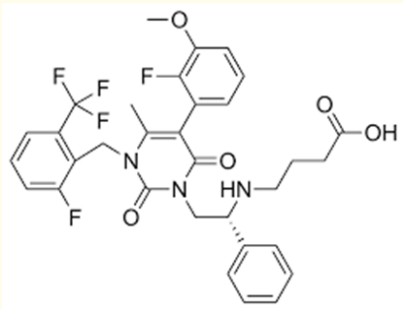


Figure 1: Chemical structure of Elagolix.

Desai., *et al.* developed a stability indicating LC-MS compatible chromatographic method [4] for the quantification of potential organic impurities of Elagolix sodium in tablet dosage form with identification of major degradation products using ACE C18-PFP column by employing a mixture of acetate buffer (pH 5.6) and acetonitrile (95: 5) as mobile phase A, and acetonitrile: methanol (90: 10) as mobile phase B with UV detection at 210 nm on gradient mode with flow rate 1.3 mL/min.

Qiong Wang., *et al.* developed a UPLC-MS/MS assay method [5] (Gradient mode) in Multiple reaction monitoring (MRM) positive ion mode in presence of an internal standard, Diazepam for the quantification Elagolix in rat plasma using Acquity UPLC BEH C18 column (PDA detector) and mobile phase consisting of a mixture of acetonitrile and 0.1% formic acid and the linearity was found to be 1-2000 ng/ml.

Yajie Hao., *et al.* developed a UPLC-MS/MS assay method [6] (Gradient mode) in for the quantification Elagolix and its genotoxic

impurities trimethyl phosphate and tri isopropyl phosphate using Shim-pack Scepter C18-120 column (PDA detector) and mobile phase consisting of a mixture of methanol and 0.1% formic acid (68:32) with flow rate 0.2 ml/min and the linearity was found to be 0.24-1.8 ng/ml.

Xueni Zhong., *et al.* developed a stability indicating high-resolution LC-ESI-MS method [7] for the estimation of Elagolix sodium and its related substances on gradient mode using Waters XBridge C18 column (PDA detector) and mobile phase consisting of a mixture of 15 mM ammonium acetate with acetonitrile/methanol (35: 65) with flow rate 1.5 ml/min and detection wavelength at 275 nm were employed for the study.

Pradip and Purnima developed a stability indicating HPLC method [8] for the estimation of Elagolix sodium in tablet dosage form with identification of major degradation products using quality by design approach. Chromatographic conditions such as Inertsil ODS-3 C18 column (PDA detector) with mobile phase consisting of 0.05% trifluoroacetic acid: acetonitrile (55:45) (Gradient mode) and flow rate of 1 ml/min and detection wavelength at 275 nm were employed for the study and the linearity was found to be 1-3 µg/ml.

Bommi., *et al.* developed a stability-indicating UHPLC method [9] in the quantification of related substances and degradation products of Elagolix sodium by Quality by Design driven approach using ultra-high performance liquid chromatography on gradient mode using Waters X-select phenyl hexyl UHPLC column (PDA detector) and mobile phase consisting of a mixture of 10 mM diammonium hydrogen orthophosphate (pH 10) and acetonitrile with flow rate 0.4 ml/min and detection wavelength at 210 nm were employed for the study and the linearity was found to be 1.2-12 µg/ml.

In the present study a new RP-HPLC method has been for the determination of Elagolix and 4 Impurities (IMP) and the method was validated as per ICH guidelines.

Materials and Methods

Elagolix and four impurities such as IMP-1, IMP-2, IMP-4 and IMP-5 were of AR grade. Analytical balance (Sartorius), Waters Alliance HPLC system with PDA/UV detector (Model No. 2996 and 2695), and Digital Ultra Sonicator (Equitron) were used for the study. Sulfuric acid (Fischer Scientific), Acetonitrile (Honeywell) (HPLC grade), Milli-Q water (HPLC grade), 0.45 µm Millipore filters were used for the entire study.

Procedure

Preparation of mobile phase

0.55 ml of Sulfuric acid was pipetted out and diluted to10 ml with water in a volumetric flask and mixed well. A mixture of dilute sulfuric acid, water and Acetonitrile was prepared in a ratio 0.4: 6.6: 93 % (v/v/v), sonicated, filtered through 0.45 µm membrane filter paper.

Preparation of Standard stock solution

Each 3.0 mg of Elagolix IMP-1, Elagolix IMP-2, Elagolix IMP-4, Elagolix IMP-5 and 2.0 mg of Elagolix standard were weighed accurately and transferred into a 100 ml volumetric flask dissolved and made up to the volume with the diluent.

Preparation of standard solution

1.0 ml of standard stock solution was transferred into 20 ml volumetric flask and made up to volume with diluent.

Test preparation

Each 10.0 mg of each of Elagolix IMP-1, Elagolix IMP-2, Elagolix IMP-4, Elagolix IMP-5 and Elagolix standard samples were accurately weighed and transferred into a 10 ml volumetric flask, dissolved and made up to volume with the diluent.

Optimized chromatographic conditions

Chromatographic experiments were performed on a Waters alliance Model HPLC system, and the separation was carried out on an Inertsil C8, (250 ×4.6 mm, 5 µ) analytical column and column temperature was 35ºC. A mixture of phosphate buffer solution (pH adjusted to 3.5 with ortho phosphoric acid) and Acetonitrile was used as mobile phase for the chromatographic study (Detection wavelength: 275 nm). Gradient elution was carried out with a flow rate of 1.0 mL/min. The total run time was 40 min.

Method validation [10-12]

Linearity, Precision and Accuracy studies

Solutions containing 20-100 µg/ml Elagolix API and its four impurities such as Elagolix impurity-1, Elagolix impurity-2, Elagolix impurity-4 and Elagolix impurity-5 were prepared from their stock solutions and injected into the HPLC system (n = 3) and the cor-

responding chromatograms were recorded. The peak area of the chromatograms of Elagolix API and its four impurities were noted and calibration curves were drawn by plotting the concentration of Elagolix API, Elagolix IMP-1, Elagolix IMP-2, Elagolix IMP-4 and Elagolix IMP-5 (IMP-1, IMP-2, IMP-3 IMP-4) solutions on the x-axis and the corresponding mean peak area on the y-axis.

Solutions of Elagolix API and its four impurities such as Elagolix impurity-1, Elagolix impurity-2, Elagolix impurity-4 and Elagolix impurity-5 (n = 6) were injected in to the HPLC system and the peak areas were noted from the chromatograms obtained and the % RSD was calculated.

Accuracy was studied by spiking the Elagolix API and its four impurities such as Elagolix impurity-1, Elagolix impurity-2, Elagolix impurity-4 and Elagolix impurity-5 solutions with the formulation and injected in to the HPLC system and the peak areas were noted from the chromatograms obtained and the % RSD was calculated.

Results and Discussion

A new validated RP-HPLC method has been developed for the quantification of Elagolix API, Elagolix IMP-1, Elagolix IMP-2, Elagolix IMP-4 and Elagolix IMP-5 (IMP-1, IMP-2, IMP-3 IMP-4) using Waters Alliance system with Inertsil C8, (250 ×4.6mm, 5µm) analytical column (PDA detector) was used for the present study. Chromatographic experiments were performed on a Waters alliance Model HPLC system, and the separation was carried out on an Inertsil and column temperature was 35ºC. A mixture of phosphate buffer solution (pH adjusted to 3.5 with ortho phosphoric acid) and Acetonitrile was used as mobile phase for the chromatographic study (Detection wavelength: 275 nm). The flow rate was 1.0 mL/min and the total run time was 40 min. Some of the parameters of the previously developed analytical methods for the estimation of Elagolix were summarised in Table 1 and the gradient program was shown in Table 2.

Mobile phase (v/v)	Method/Column	λ (nm)	Comment	Ref
Mobile phase A: Acetate buffer (pH 5.6): Acetonitrile (95:5) Mobile phase B: Acetonitrile: Methanol (90: 10)	LC-MS ACE C18-PFP	210	Potential organic impurities (Gradient mode)	[4]
Acetonitrile: 0.1% Formic acid	UPLC-MS/MS Acquity UPLC BEH C18	-	Rat plasma (Gradient mode) Diazepam (Internal standard)	**[5]**
Methanol: 0.1% Formic acid (68:32)	UPLC-MS/MS Shim-pack Scepter C18-120	--	Genotoxic impurities (Gradient mode)	[6]
15 mM Ammonium acetate: Acetonitrile/Methanol (35: 65)	LC-ESI-MS XBridge C18	275	Related substances (Gradient mode)	[7]
0.05% Trifluoro acetic acid: Acetonitrile (55:45)	RP-HPLC Inertsil ODS-3 C18	275	QbD (Gradient mode)	[8]
10 mM Diammonium hydrogen ortho phosphate (pH 10): Acetonitrile	RP-UHPLC X-select phenyl hexyl C18	210	QbD (Gradient mode)	[9]
Phosphate buffer (pH 3.5 with ortho phosphoric acid): Acetonitrile	RP-HPLC Inertsil C8	275	4 Impurities (Gradient mode)	Present method

Table 1: Literature survey.

Time (min)	Solution-A (%)	Solution-B (%)
0.0	70.0	30.0
5.0	70.0	30.0
20.0	25.0	75.0
25.0	0.0	100.0
30.0	0.0	100.0
35.0	70.0	30.0
40.0	70.0	30.0

Table 2: Gradient program.

Method validation

Linearity, Precision and Accuracy

Linearity was shown over the concentration range 20-100 µg/ml for Elagolix API, Elagolix IMP-1, Elagolix IMP-2, Elagolix IMP-4 and Elagolix IMP-5 (Table 3) and the LOQ results were shown in Table 4 (Figure 2).

The representative chromatograms of Elagolix API, Elagolix IMP-1, Elagolix IMP-2, Elagolix IMP-4 and Elagolix IMP-5 (IMP-1, IMP-2, IMP-3 IMP-4) were shown in Figure 3. Calibration curves were drawn by taking the concentration of Elagolix API, Elagolix IMP-1, Elagolix IMP-2, Elagolix IMP-4 and Elagolix IMP-5 (IMP-1, IMP-2, IMP-3 IMP-4) on the x axis and the corresponding peak area on the y axis (Figure 4) with the linear regression equations y = 788.61x + 16037 (R^2 = 0.9986), y = 1644.6x + 32081 (R^2 = 0.9469), y = 291.63x + 8636.3 (R^2 = 0.9971), y = 2992.8x + 8708.7 (R^2 = 0.9988) and y = 1600.3x + 17559 (R^2 = 0.9653) respectively.

Conc. (µg/ml)	Elagolix API	Elagolix IMP-1	Elagolix IMP-2	Elagolix IMP-4	Elagolix IMP-5
20	30657	56601	13999	65589	41812
30	40150	77115	17852	98300	63053
50	55767	120208	23759	161334	103719
65	68466	148737	27019	203519	128781
75	75361	168800	30427	236883	147075
100	93948	180176	37916	304189	165027
Linear regression equation	y = 788.61x + 16037 (R^2 = 0.9986)	y = 1644.6x + 32081 (R^2 = 0.9469)	291.63x + 8636.3 (R^2 = 0.9971)	y = 2992.8x + 8708.7 (R^2 = 0.9988)	y = 1600.3x + 17559 (R^2 = 0.9653)

Table 3: Linearity of Elagolix and impurities.

Injection	Limit of quantification				
	Peak area				
	Elagolix Imp-1	Elagolix	Elagolix Imp-2	Elagolix Imp-4	Elagolix Imp-5
1	53145	30579	12163	65387	41932
2	51009	30489	12780	64265	41797
3	52453	29920	14987	66180	41955
4	52763	30774	11568	65058	41928
5	52394	29939	14195	66687	41979
6	52354	30267	14695	65375	41631
Average	52353	30328	13398	65492	41870
% RSD	1.26	1.05	9.68	1.19	0.29

Table 4: Limit of quantification (LOQ).

The precision results of Elagolix API and its four impurities such as Elagolix impurity-1, Elagolix impurity-2, Elagolix impurity-4 and Elagolix impurity-5 were shown in Table 5. The accuracy of the method was proved by checking the recovery of known impurities. Test solution was spiked with known impurities at LOQ, 50%, 100% and 200% level.

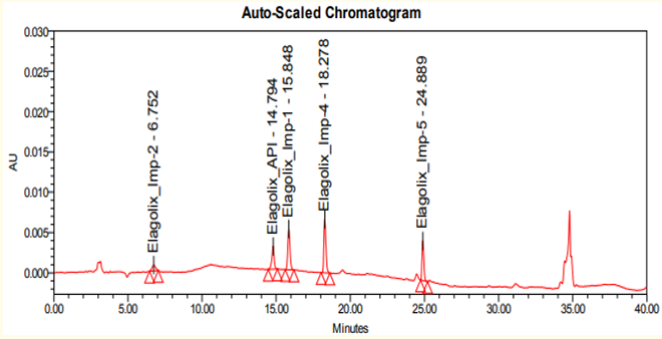
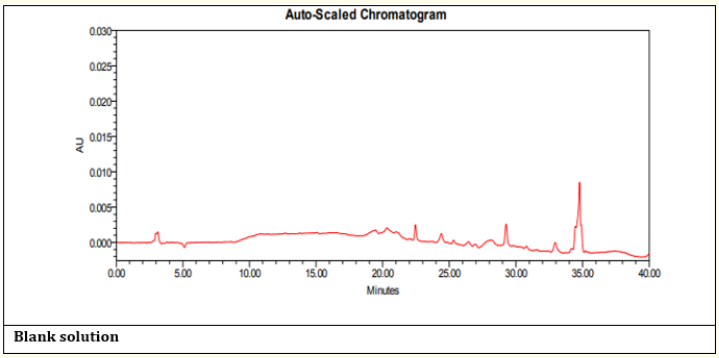
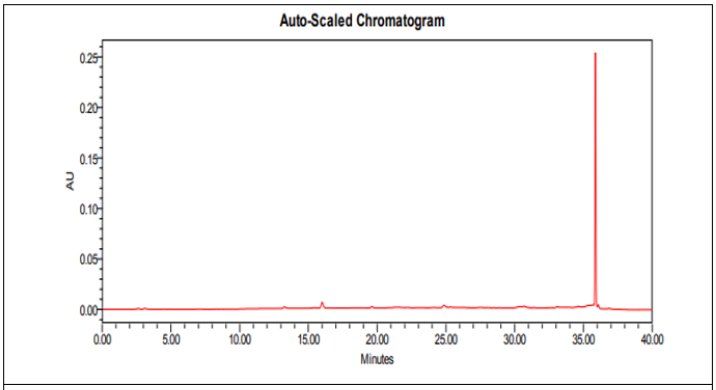


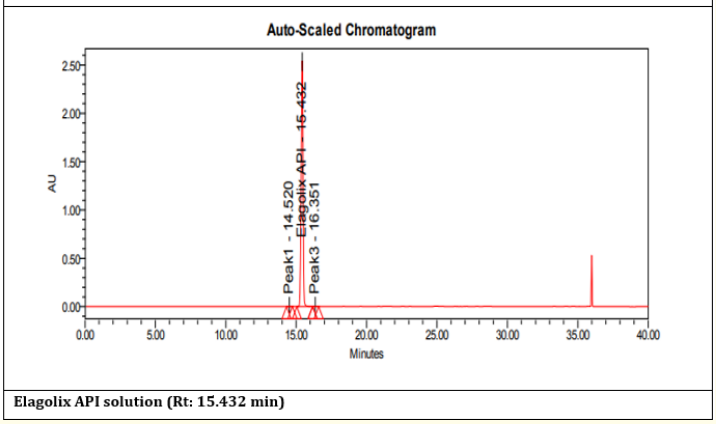
Figure 2: Chromatogram for LOQ solution.



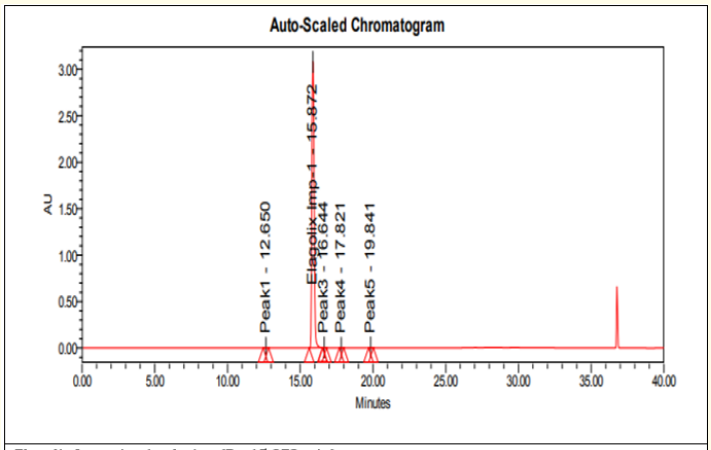
Blank solution



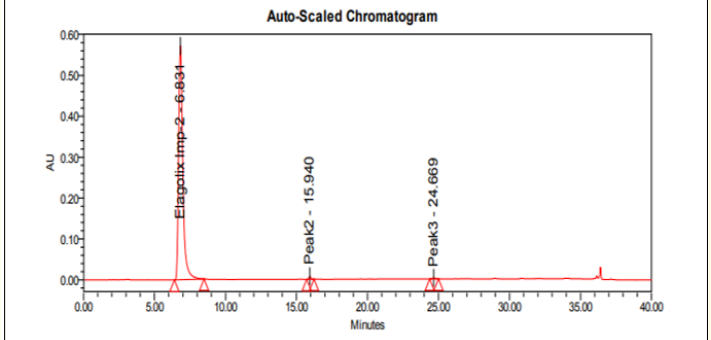
Placebo solution



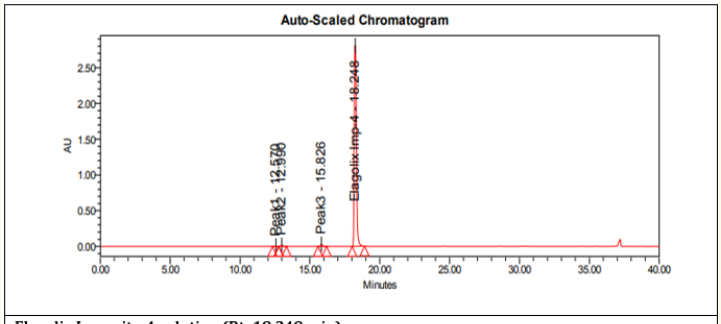
Elagolix API solution (Rt: 15.432 min)



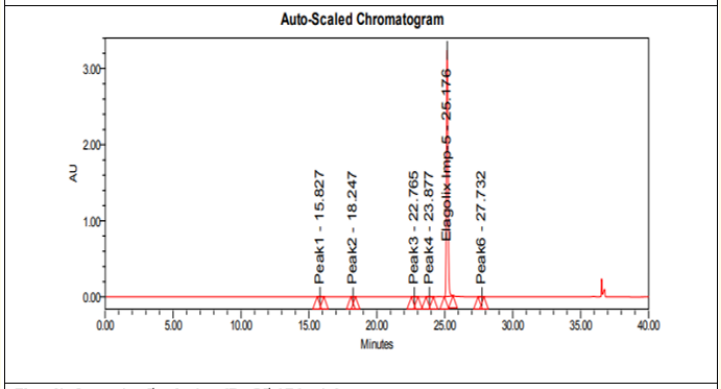
Elagolix Impurity-1 solution (Rt: 15.872 min)



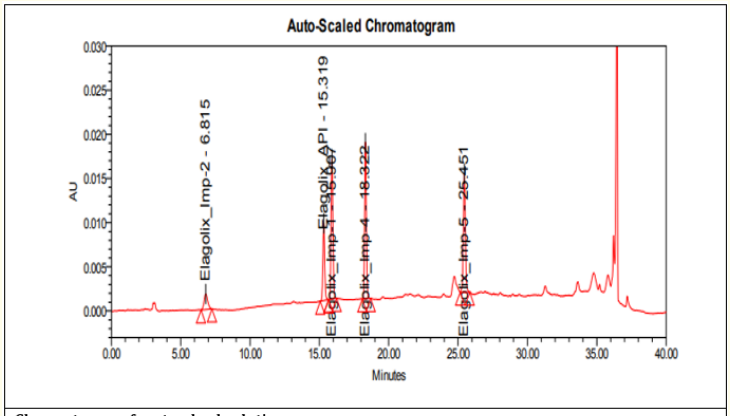
Elagolix Impurity-2 solution (Rt: 2.6831 min)



Elagolix Impurity-4 solution (Rt: 18.248 min)



Elagolix Impurity-5 solution (Rt: 25.176 min)

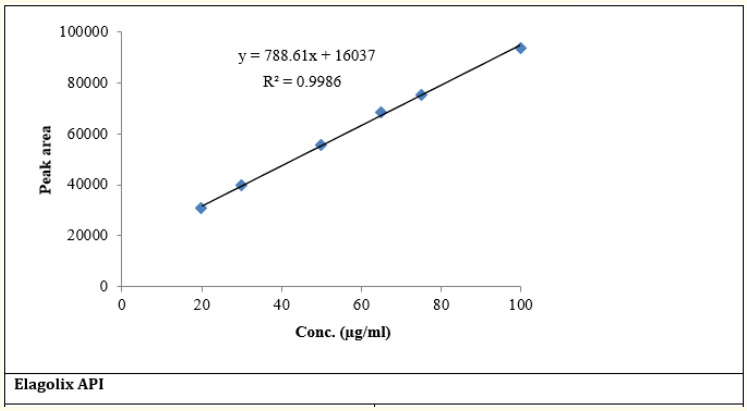


Chromatogram for standard solution

Figure 3: Representative chromatograms of Elagolix and its impurities.

Peak area					
S. No.	**Elagolix Imp-2**	**Elagolix**	**Elagolix Imp-1**	**Elagolix Imp-4**	**Elagolix Imp-5**
1	32368	75305	141356	146645	140060
2	32502	74129	141579	147818	142712
3	32390	74596	140973	147429	140642
4	32747	75730	141134	147375	144404
5	32389	74292	140227	146279	141534
6	32842	74245	139657	144095	142933
Average	32540	74716	140821	146607	142047
Std. dev.	187.18	596.59	669.56	1234.94	1470.17
% RSD	.58	0.80	0.48	0.84	1.03

Table 5: Precision study.



Elagolix API

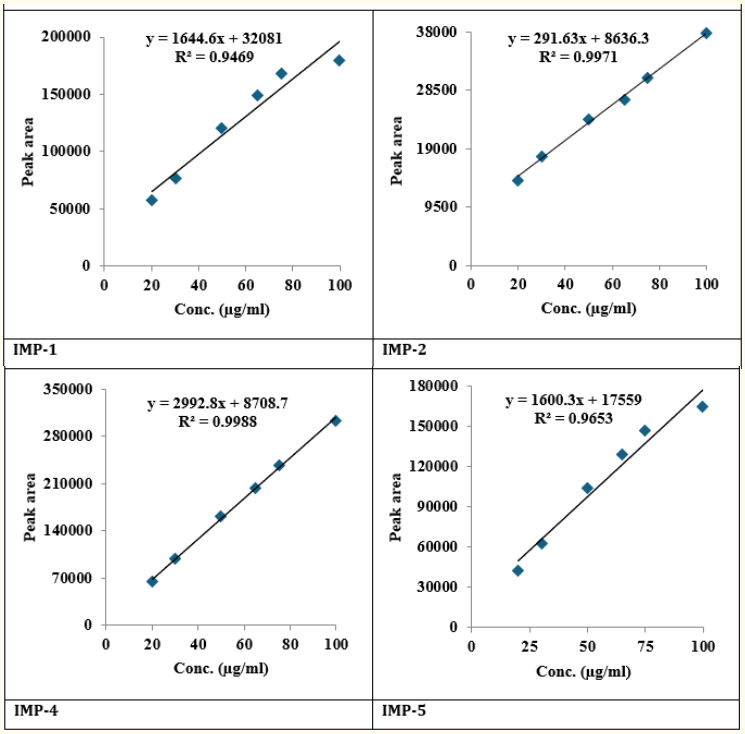


Figure 4: Calibration curves of Elagolix and impurities (IMP).

Conclusion

A simple, rapid, accurate and precise RP-HPLC method has been developed for the quantification of Elagolix API and its four impurities such as Elagolix impurity-1, Elagolix impurity-2, Elagolix impurity-4 and Elagolix impurity-5 and the method was validated as per ICH guidelines and the method is suitable for the routine analysis.

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