ISSN: 2231-3656

International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol. 16 | Issue 4 | Oct – Dec 2026

www.ijpir.com

DOI: https://doi.org/10.61096/ijpir.v16.iss4.2026.1092-1105

Analytical Method Development and Validation for Elagolix and its Related Substances: A Comprehensive Review

Samyuktha, *P. Vivek Sagar, J. Sunayana, S. Muni Sirisha

Department of Pharmaceutical Analysis, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya (Co-Ed.), Tarnaka, Secunderabad, Telangana, India

Corresponding author: ***Dr. P. Vivek Sagar**
Email: dr.p.viveksagar@gmail.com.

Published on: 13.11.2026
Published by: Futuristic Publications
2026| All rights reserved.

Creative Commons Attribution 4.0 International License.

Abstract: Elagolix sodium is the first orally active, non-peptide gonadotropin-releasing hormone (GnRH) receptor antagonist, approved by the US FDA in 2018 for moderate to severe endometriosis-associated pain (Orilissa® tablets, 150 and 200 mg) and later in combination with estradiol and norethindrone acetate for heavy menstrual bleeding due to uterine fibroids (Oriahnn® capsules). Its uracil core carries a secondary-amine, butanoic-acid side chain, a chiral centre and hindered biaryl rotation, which together give a varied impurity profile of process intermediates, the S-enantiomer, atropisomers, oxidative and cyclised (δ-lactam) degradants, potential N-nitroso derivatives and alkyl-phosphate genotoxic impurities.

This review surveys the analytical methods published for elagolix between 2020 and 2026. It covers RP-HPLC assay methods, stability-indicating related-substances methods by HPLC and UHPLC, chiral and atropisomer separations, LC-MS/MS methods for genotoxic impurities and plasma, structural elucidation of degradation products, spectrofluorimetric methods, and a method for the fixed-dose combination. For each, the chromatographic conditions, validation findings and limitations are summarised.

Although several related-substances methods now exist, they disagree on degradation behaviour (alkaline lability), use long runs (46–75 min) or extreme pH, rarely quantify identified degradants against reference standards, and none is pharmacopoeial or validated under ICH Q2(R2)/Q14. N-nitroso elagolix and atropisomer control remain largely unaddressed. A strategy for a robust, MS-compatible, stability-indicating method is proposed.

Keywords: Elagolix sodium; related substances; stability-indicating method; RP-HPLC; UHPLC; forced degradation; quality by design; ICH Q2(R2)

1. Introduction

Endometriosis affects roughly one in ten women of reproductive age and causes chronic pelvic pain, dysmenorrhoea and infertility. Hormonal suppression is the mainstay of medical treatment. Injectable peptide GnRH agonists suppress oestrogen effectively but cause an initial flare and profound hypo-oestrogenism. Elagolix was designed as an oral, short-acting, non-peptide GnRH receptor antagonist that gives rapid, dose-dependent and reversible suppression of gonadotropins and ovarian hormones [1,4].

The US FDA approved elagolix sodium (Orilissa®, AbbVie) in July 2018 as 150 mg once-daily and 200 mg twice-daily tablets for endometriosis-associated pain [2]. A capsule co-packaging elagolix 300 mg with estradiol 1 mg and norethindrone acetate 0.5 mg (Oriahnn®) followed in 2020 for heavy menstrual bleeding due to uterine fibroids [3]. Generic development has since begun in several markets, which has driven much of the recent analytical literature.

From an analytical standpoint elagolix is demanding. The molecule contains a uracil core, three fluorinated aromatic rings, a secondary amine, a carboxylic acid and a stereocentre [4,5]. Restricted rotation about the aryl–uracil bond produces slowly interconverting atropisomers [5,28]. The amine and acid can cyclise, the amine can be oxidised or nitrosated, and the synthesis uses reagents that leave potentially genotoxic residues [20,23]. A related-substances method must therefore resolve structurally similar process impurities, degradants and stereoisomers from a zwitterionic parent that is formulated as a hygroscopic amorphous sodium salt [1].

ICH Q3A(R2) and Q3B(R2) require impurities in the drug substance and degradation products in the drug product to be reported, identified and qualified above defined thresholds [6,7]. ICH M7(R2) and regulatory guidance on nitrosamine drug substance-related impurities (NDSRIs) add limits in the parts-per-million range for mutagenic impurities [8,13]. Meeting these requirements needs validated, stability-indicating methods.

This review collates the analytical work published on elagolix to date. It summarises the physicochemical profile and impurity landscape, the regulatory position, and each published method with its conditions, findings and limitations. It then identifies the gaps and sets out the scope for developing and validating an improved method for elagolix and its related substances.

2. Drug profile and impurity landscape

2.1 Drug profile

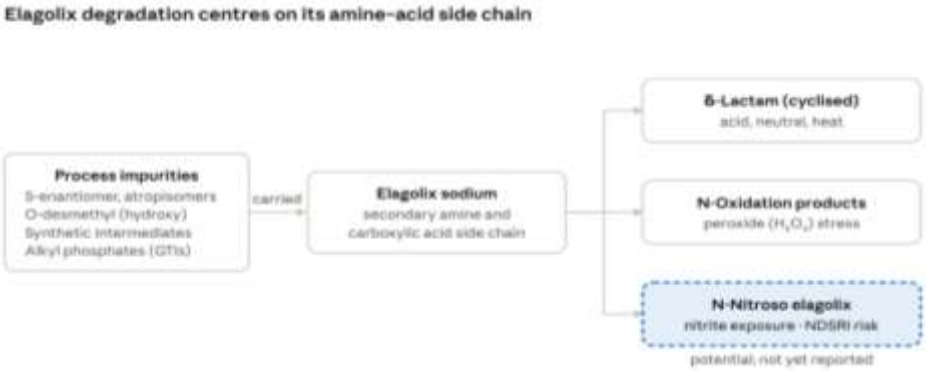
Table 1. Physicochemical profile of elagolix sodium

Property	Description
Chemical name	Sodium 4-({(1R)-2-[5-(2-fluoro-3-methoxyphenyl)-3-{[2-fluoro-6-(trifluoromethyl)phenyl]methyl}-4-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl]-1-phenylethyl}amino)butanoate
Molecular formula	$C_{32}H_{29}F_5N_3NaO_5$ (free acid $C_{32}H_{30}F_5N_3O_5$)
Molecular weight	653.6 g/mol (sodium salt); 631.6 g/mol (free acid)
Description	Hygroscopic, amorphous, white to off-white powder; no polymorphism reported
Ionisation	Zwitterion; pKa about 4.0 (carboxylic acid) and 7.9 (secondary amine) [1]
Solubility	High aqueous solubility (about 1 mg/mL) across physiological pH; BCS class III [1]
Stereochemistry	One stereocentre (R); hindered aryl–uracil rotation gives two atropisomers [5,28]
UV absorption	Detection wavelengths of 210 nm (impurities) and 275 nm (assay) used in published methods [14,21,22]
Mass spectrometry	$[M+H]^+$ m/z 632.4; product ions m/z 529.5 and 177.1 [16]
Marketed products	Orilissa® tablets 150 mg and 200 mg; Oriahnn® capsules (elagolix 300 mg with estradiol 1 mg and norethindrone acetate 0.5 mg) [2,3]

2.2 Impurity landscape

Elagolix impurities arise from three sources. Process impurities include unreacted intermediates, the S-enantiomer, the O-desmethyl (2-fluoro-3-hydroxyphenyl) analogue and residual alkyl phosphates such as trimethyl phosphate and triisopropyl phosphate, which are potential mutagens [22,23,25]. Degradation products form mainly on the 4-aminobutanoic acid side chain [20]. Under acidic, neutral and thermal stress the acid and secondary amine cyclise to a δ-lactam; peroxide oxidises the amine to an N-hydroxylamine,

which breaks down further to amine and carbonyl analogues [18,20]. A secondary amine is also a structural alert for nitrosation, so N-nitroso elagolix is a credible NDSRI risk, and reference standards for it are commercially available [13].



Sources of elagolix related substances compiled from [5,18,20–23]

The uracil core is stable under all stress conditions reported [20]. Reports disagree on alkaline stability: Zhong et al. found no significant base degradation [20], whereas Desai et al. reported significant degradation under alkali hydrolysis in the tablet [21]. This difference may reflect formulation effects or stress severity, and it affects which degradants a method must resolve.

3. Regulatory and pharmacopoeial status

Elagolix sodium has no monograph in the USP–NF, Ph. Eur., BP, JP or IP editions checked for this review **[verify current editions before submission]**. No official related-substances procedure or compendial impurity limits therefore exist. Specified impurities and limits are set by each applicant in the drug master file or marketing application and are not public. Generic developers must design their impurity control strategy from ICH guidance and the published literature.

Table 2. Regulatory framework applicable to elagolix related substances

Guideline	Requirement relevant to elagolix	Practical implication
ICH Q3A(R2) [6]	Reporting 0.05%, identification 0.10% and qualification 0.15% thresholds for a maximum daily dose ≤ 2 g	Drug-substance methods need LOQ ≤ 0.05% of the test concentration
ICH Q3B(R2) [7]	Degradation-product thresholds for the drug product; at 400 mg/day (200 mg twice daily) the reporting threshold is 0.1% and identification 0.2%	Tablet and capsule methods must quantify degradants at ≤ 0.1%
ICH M7(R2) [8]	Mutagenic impurities controlled to the threshold of toxicological concern (1.5 µg/day lifetime) or compound-specific limits	Alkyl phosphates need trace methods (ppm level) [23]
FDA and EMA nitrosamine guidance [13]	NDSRIs assessed by risk; acceptable intakes assigned by structural potency category	N-nitroso elagolix needs a risk assessment and, if required, a sensitive LC-MS/MS confirmatory test
ICH Q1A(R2), Q1B [9,10]	Stress testing and photostability	Forced degradation to demonstrate the method is stability-indicating
ICH Q2(R2), Q14 [11,12]	Validation and science- and risk-based development (2023)	Analytical target profile, design of experiments, method operable design range

The maximum daily dose of 400 mg (Orilissa) or 600 mg (Oriahnn, two 300 mg capsules) places elagolix in the band where degradant identification is required at 0.2% (or 2 mg total daily intake, whichever is lower) [7]. With a test concentration near 0.5–1.2 mg/mL, as used in published methods, a reporting threshold of 0.1% corresponds to roughly 0.5–1.2 µg/mL, which is within the sensitivity reported by most UV methods [21,22].

4. Review of published analytical methods

About 17 analytical reports on elagolix have appeared since 2020. They fall into six groups: RP-HPLC assay methods, stability-indicating related-substances methods (HPLC and UHPLC), chiral and atropisomer separations, structural elucidation of degradants, trace LC-MS/MS methods for genotoxic impurities, and bioanalytical or spectrofluorimetric methods. Table 3 summarises each in chronological order.

Table 3. Published analytical methods for elagolix and its related substances

#	Author, year [ref]	Purpose and matrix	Column and mobile phase	Detection	Key findings	Observations and limitations
1	Choppari et al., 2020 [15]	Enantiomeric purity; drug product	CHIRALPAK ZWIX(+) zwitterionic CSP; 50 mM acetic acid + 25 mM diethylamine in water–methanol (2:98); 0.5 mL/min; isocratic	UV	Baseline separation of the S-enantiomer; LOD 2.0 ng/mL, LOQ 7.5 ng/mL for the enantiomer	First chiral method. Atropisomers not discussed; polar-ionic mobile phase not transferable to achiral impurity method
2	Todkar and Hamrapurkar, 2021 [14]	Stability-indicating assay (QbD); API	Inertsil ODS-3 C18, 250 × 4.6 mm, 5 µm; 0.05% TFA–acetonitrile (55:45); 1.0 mL/min	PDA 275 nm	Rt 9.06 min; linear 1–3 µg/mL (r^2 0.9992); LOD 0.2, LOQ 0.5 µg/mL; recovery 100.75%; RSD < 0.9%; degradation 0.8% acid, 0.62% base, none under oxidation, heat or light	Assay only; very narrow range; mobile-phase ratio reported inconsistently; mild stress missed the oxidative lability found later [18,20]
3	Wang et al., 2021	Pharmacokinetics; rat	Acquity UPLC BEH	ESI+ MRM m/z	Linear 1–2000 ng/mL	Bioanalytical; defines MS

	[16]	plasma	C18, 2.1 × 100 mm, 1.7 µm; acetonitrile–0.1% formic acid gradient; protein precipitation; diazepam IS	632.4 → 529.5	(r^2 > 0.9983); intra-day RSD 3.5–5.5%, inter-day 9.4–12.7%	transitions useful for impurity identification
4	Rasha M. Ahmed, 2021 [17]	Assay, content uniformity, plasma	Micellar spectrofluorimetry	Fluorescence	Green, low-cost determination in tablets and plasma	Not separative; cannot assess related substances
5	Kotha et al., 2022 [18]	Forced degradation; drug substance	Gradient LC-MS; preparative HPLC isolation	HRMS, NMR	Three novel oxidative degradants isolated and characterised; stable to acid, base, neutral, heat and light	Structural study; no validated quantitative method
6	Salman et al., 2022 [19]	Plasma and content uniformity	Boron-doped carbon quantum dots (B@CQDs)	Fluorescence enhancement	Linear 4–100 ng/mL; LOQ 1.74 ng/mL; applied to a human pharmacokinetic study	Not stability-indicating
7	Zhong et al., 2023 [20]	Forced degradation and mechanisms; drug substance	XBridge C18; 15 mM ammonium acetate with acetonitrile–methanol	LC-UV-PDA-MSn, NMR	Seven new degradants; degradation centred on the side-chain amine and acid; δ-lactam under acid/neutral/heat; N-hydroxylamine and further products	Most complete degradation map. Characterisation only; artefact degradants must be excluded from specifications

					under H_2O_2; stable to alkali; two degradants form only in solution	
8	Desai et al., 2023 [21]	Related substances; tablets	ACE C18-PFP, 250 × 4.6 mm, 5 µm; A: acetate pH 5.6–acetonitrile (95:5); B: acetonitrile–methanol (90:10); gradient, 46 min; 1.3 mL/min	UV 210 nm; MS	All prominent organic impurities resolved; LOD 0.08 µg/mL; significant alkali and oxidative degradation; mass balance > 97%; validated to ICH Q2(R1)	LC-MS compatible. Long run; alkali finding conflicts with [20]
9	Bommi et al., 2023 [22]	Related substances and intermediates (QbD); drug substance	XSelect phenyl-hexyl UHPLC; 10 mM diammonium hydrogen phosphate pH 10.0–acetonitrile gradient; 0.4 mL/min; 40 °C	UV 210 nm	Nine impurities including precursor intermediates; accuracy 91.8–107.2%; RSD 0.9% (elagolix, 1.2 mg/mL) and 0.8–1.8% (impurities, 0.5 µg/mL); method operable design range defined	Fast and QbD-based. pH 10 stresses silica columns; non-volatile phosphate prevents LC-MS; drug substance only; thermal and photolytic stress not reported
10	Ciceri S et al., 2023 [5]	Structure, conformation and atropisomers; API and	Cellulose-based chiral HPLC	UV, NMR, X-ray	Atropisomers separated; literature synthesis gave only 86% HPLC	Characterisation; no validated method

		intermediates			purity, highlighting process-impurity burden	
11	Hao et al., 2024 [23]	Genotoxic impurities trimethyl and triisopropyl phosphate; API	Shim-pack Scepter C18-120; methanol–0.1% formic acid (68:32)	UHPLC-MS/MS	LOQ 24 pg/mL, LOD 4.8 pg/mL; 6 min run; validated to ICH Q2 and M7	Covers two GTIs only; N-nitroso derivatives not included
12	Kulkarni et al., 2024 [24]	Elagolix and four impurities; API	Inertsil C8, 250 × 4.6 mm, 5 µm; 35 °C; phosphate pH 3.5–acetonitrile gradient, 40 min; 1.0 mL/min	PDA 275 nm	Linear 20–100 µg/mL for all analytes (r^2 0.947–0.999); repeatability RSD 0.48–1.03%	Impurity linearity tested at assay level, not at 0.05–0.15%; r^2 below 0.99 for two impurities; LOD, LOQ, recoveries and stress results not reported; impurity structures not given
13	Aline Augusti Boligon, 2024 [25]	Related substances of synthetic intermediate 6	ReproSil C18, 250 × 4 mm, 5 µm; 0.1% H_3PO_4–methanol gradient; 1.1 mL/min; 25 °C	UV 215 nm	Intermediate 6 separated from other intermediates; LOQs meet control limits	In-process control; supports the purge argument for intermediates
14	Bandaru et al., 2024 [26]	Related substances (QbD robustness); tablets 150 and 200 mg	RP column **[verify]**; A: 10 mM NaH_2PO_4 pH 6.0–acetonitrile (95:5); B:	UV 210 nm	Linear LOQ–200%; accuracy LOQ–150%; r > 0.9999 for elagolix; DoE-verified	75 min run; phosphate buffer not MS-compatible; photolysis

			acetonitrile–water–methanol (85:10:5); 75 min		robustness; stable to hydrolysis, oxidation and heat as tested	not reported
15	Saroja et al., 2025 [27]	Elagolix, estradiol and norethindrone; Oriahnn capsules	RP-HPLC, isocratic	DAD 248 nm	Rt 3.21, 5.15 and 6.82 min; r^2 > 0.999; RSD 0.09–0.71%; recovery 99.13–101.32%; LOD 0.008–0.854 µg/mL; peak purity under stress	Assay only; impurities of the three actives not resolved or quantified
16	Nader A, 2026 [28]	Atropisomer separation and interconversion	Polysaccharide CSPs by SFC	UV	Rs > 2.0 within 15 min; rotational barriers 23–26 kcal/mol, half-lives minutes to days (LaPlante class II)	Atropisomer standards unavailable; only total elagolix quantified
17	AbbVie, clinical studies [29]	Pharmacokinetics; human plasma	LC-MS/MS after salt-assisted protein precipitation	MS/MS	LLOQ 1.57 ng/mL	Method details not published

5. Critical observations

5.1 Stationary-phase choice has moved beyond plain C18. Plain C18 and C8 columns serve for assay [14,24], but the related-substances methods that resolve the most impurities use alternative selectivity: pentafluorophenyl-C18 [21] and phenyl-hexyl [22]. Elagolix and its impurities carry three fluorinated aromatic rings, and π–π and dipole interactions on these phases separate positional and des-methyl analogues that co-elute on C18.

5.2 Mobile-phase pH spans 3.5 to 10.0 with no consensus. Published related-substances methods use pH 3.5 phosphate [24], pH 5.6 acetate [21], pH 6.0 phosphate [26] and pH 10.0 ammonium phosphate [22]. Because elagolix is a zwitterion (pKa 4.0 and 7.9), retention and peak shape change sharply between these values [1]. Operating near either pKa invites robustness failures, which is why the QbD studies emphasise pH as a critical parameter [22,26].

5.3 Run times are long except on UHPLC. HPLC related-substances methods need 40–75 min [21,24,26], while the UHPLC method separates nine impurities in a fraction of that time at 0.4 mL/min [22]. Long gradients reduce throughput for stability programmes and release testing.

5.4 Degradation behaviour is reported inconsistently. All studies agree that elagolix is sensitive to oxidation [18,20,21]. They disagree on hydrolysis: one structural study found the drug stable to alkali [20], another found it stable to all but oxidation [18], and a tablet method reported significant alkaline degradation [21]. An early assay method found almost no degradation under any condition [14]. These differences point to non-harmonised stress conditions and to excipient effects, and they make it hard to know which degradants a specification should list.

5.5 Identification and quantification are rarely linked. Two studies characterised degradants by HRMS and NMR [18,20] but did not validate a quantitative method, while the validated methods identify impurities by code (IMP-1, IMP-2) or by vendor names without structures [24,26]. Only Desai et al. combined a validated method with MS identification of major degradants [21]. Zhong et al. showed that two degradants form only in solution and are not true product impurities, a distinction that matters for specifications [20].

5.6 Validation quality varies widely. Strong methods report LOQ–150% accuracy, DoE robustness and mass balance [21,22,26]. Others validate impurity linearity at assay concentrations, report r^2 below 0.99, or omit LOQ, recovery and stress data [24]. All validated methods follow ICH Q2(R1); none applies Q2(R2) or Q14, both adopted in 2023 [11,12].

5.7 Mutagenic impurities are only partly covered. A 6 min UHPLC-MS/MS method controls trimethyl and triisopropyl phosphate at picogram-per-millilitre levels [23]. No published method addresses N-nitroso elagolix, despite the secondary amine and regulatory focus on NDSRIs [13].

5.8 Stereochemical purity is handled separately. The S-enantiomer is controlled by a dedicated chiral method [15], and atropisomers by polysaccharide CSPs under HPLC or SFC [5,28]. Atropisomer interconversion on the timescale of minutes to days can broaden or split the elagolix peak in achiral methods if column temperature and run time are not controlled [28].

6. Gaps identified

No single published method yet offers a fast, MS-compatible, stability-indicating procedure that quantifies the identified process impurities and degradants of elagolix in the tablet, validated to current ICH guidance. Table 4 lists the specific gaps.

Table 4. Gaps in the literature and the improvement each calls for

#	Gap	Evidence	Improvement needed
1	No pharmacopoeial monograph or public impurity specification	No USP/Ph. Eur./IP monograph found	A well-documented method with named, structurally identified specified impurities
2	Long run times on HPLC	40–75 min gradients [21,24,26]	A method of ≤ 25 min on HPLC (or ≤ 10 min on UHPLC) without loss of resolution
3	Extreme or non-volatile buffers	pH 10.0 ammonium phosphate [22]; sodium phosphate [24,26]	A volatile, mid-pH buffer (ammonium formate or acetate) compatible with LC-MS and long column life
4	Conflicting degradation profiles	Alkaline lability reported in [21] but not in [18,20]	Harmonised stress design on API, placebo and tablet, with kinetics and mass balance
5	Degradants identified but not quantified against standards	Characterisation-only studies [18,20]; coded impurities [24]	Quantification of the δ-lactam, N-hydroxylamine and other confirmed degradants using standards or relative response factors
6	Solution artefacts not excluded	Degradants formed only in solution [20]	Diluent and solution-stability studies to keep artefacts out of reported results

7	Incomplete validation in some methods	Assay-level impurity linearity; missing LOQ and recovery [24]	LOQ at or below 0.05% and linearity from LOQ to 150% of each limit
8	No method for N-nitroso elagolix	NDSRI risk from the secondary amine; no publication found [13]	A trace LC-MS/MS method validated against the acceptable intake
9	Stereoisomers and atropisomers handled only by separate methods	[5,15,28]	Evaluate atropisomer behaviour in the achiral method; define temperature and integration rules
10	Combination product impurities not addressed	Oriahnn method covers assay only [27]	Related-substances method for elagolix, estradiol and norethindrone acetate in the capsule
11	No ICH Q2(R2)/Q14 validation	All validated methods use Q2(R1) [21,22,26]	Analytical target profile, risk assessment, DoE and lifecycle approach
12	Little greenness assessment for impurity methods	Greenness assessed only for spectrofluorimetry [17]	Greener solvents, shorter runs and AGREE or GAPI scoring

7. Scope and proposed strategy for a related-substances method

The literature points to a clear opportunity: combine the fluorinated-phase selectivity of Desai et al. [21] with the speed and QbD rigour of Bommi et al. [22], in a volatile mid- or low-pH mobile phase, and validate it under ICH Q14 and Q2(R2) for both drug substance and tablets [11,12].

7.1 Analytical target profile

The procedure should separate and quantify elagolix, its known process impurities (including intermediates and the O-desmethyl analogue) and its confirmed degradants (δ-lactam and N-oxidation products) in elagolix sodium and in 150/200 mg tablets. It should achieve resolution ≥ 2.0 for every critical pair, LOQ ≤ 0.05% of the test concentration, accuracy of 80–120% at the LOQ and 90–110% at the specification level, and run in ≤ 25 min on HPLC, with peak identity confirmable by LC-MS.

7.2 Proposed starting conditions

Table 5. Suggested initial chromatographic conditions, to be optimised

Parameter	Proposed starting point	Rationale
Column	Pentafluorophenyl-C18 or phenyl-hexyl, 150 × 4.6 mm, 2.7 µm core-shell (UHPLC: 100 × 2.1 mm, 1.7–1.8 µm)	Fluorinated and aromatic selectivity resolved the most impurities [21,22]
Mobile phase A	10 mM ammonium formate, pH 3.0 (screen pH 2.5–3.5); alternative 10 mM ammonium bicarbonate pH 9.5 on a hybrid column	At least one unit from both pKa values (4.0, 7.9) for robust retention; volatile for LC-MS [1]
Mobile phase B	Acetonitrile (screen 10% methanol as modifier)	Methanol fine-tunes selectivity for aromatic analogues [21,26]
Elution	Linear gradient from about 20% to 85% B over 18 min, wash and re-equilibrate	Target total run ≤ 25 min versus 46–75 min reported [21,26]
Flow and temperature	1.0 mL/min (0.4 mL/min UHPLC); 35–40 °C, tightly controlled	Fixed temperature stabilises atropisomer peak shape [28]
Detection	PDA, 210 nm for impurities and 275 nm for assay; 200–400 nm spectra for peak purity; MS for identity	Wavelengths used in published methods [14,21,22]
Diluent	Acetonitrile–water (50:50), neutral, freshly prepared	Avoids acid- or heat-driven lactam formation and solution artefacts in the vial [20]
Test concentration	0.5–1.0 mg/mL elagolix	Places 0.05% (0.25–0.5 µg/mL) above the LOQ reported by sensitive methods [21,22]

7.3 Development workflow

1. Collect reference standards for available process impurities, the S-enantiomer, the O-desmethyl analogue and the δ-lactam; generate other degradants by targeted stress and confirm by LC-MS [18,20,21].
2. Screen two or three columns (PFP, phenyl-hexyl, C18) at two pH values in a short design of experiments, scoring the critical-pair resolution.
3. Optimise gradient slope, temperature and pH by a response-surface design and define the method operable design range [22,26].
4. Optimise tablet sample preparation (diluent, sonication time, filter compatibility) using spiked placebo.
5. Run harmonised forced degradation on API, placebo and tablet, with peak purity and mass balance.
6. Validate per ICH Q2(R2) (Table 6) and apply to marketed and stability batches.

7.4 Forced degradation design

Stress API, placebo and tablet powder in parallel: acid (0.1–1 M HCl, 60 °C), base (0.1–1 M NaOH, 60 °C), neutral hydrolysis, oxidation (0.3–3% H_2O_2, room temperature), dry heat (80 °C), humidity (40 °C/75% RH) and ICH Q1B light [9,10]. Target 5–20% degradation, record kinetics, and run matching stressed placebo and solvent controls. This harmonised design should settle the conflicting reports on alkaline stability [20,21] and separate true degradants from solution artefacts [20].

7.5 Validation plan

Table 6. Proposed ICH Q2(R2) validation

Parameter	Design	Typical acceptance criterion
System suitability	Solution of elagolix spiked with all available impurities	Rs ≥ 2.0 for critical pairs; tailing ≤ 2.0; %RSD of six standard injections ≤ 5.0%
Specificity	Blank, placebo, individual impurities, spiked sample, stressed samples	No interference; peak purity passes; mass balance 95–105%
LOD and LOQ	Signal-to-noise or calibration slope per impurity	LOQ ≤ 0.05%; S/N ≥ 10 at LOQ
Linearity and range	LOQ to 150% of each specification limit, ≥ 6 levels	r ≥ 0.99 for impurities, ≥ 0.999 for elagolix
Relative response factors	Slope ratio of each impurity to elagolix	Applied where outside 0.8–1.2
Accuracy	Placebo spiked at LOQ, 50%, 100% and 150% of limit, triplicate	80–120% at LOQ, 90–110% at other levels
Precision	Six spiked preparations; second analyst, day, column lot and instrument	%RSD ≤ 10% (≤ 15% near LOQ)
Robustness	DoE on pH ±0.2, temperature ±5 °C, flow ±10%, initial %B ±2%	System suitability met across the design space
Solution stability	Standard and sample at 5 °C and 25 °C for 24–48 h	No new peaks; impurity change within agreed limits

7.6 Complementary methods

The related-substances method should be supported by three companion procedures: a trace LC-MS/MS method for N-nitroso elagolix validated against its acceptable intake [13], the existing UHPLC-MS/MS method for alkyl phosphates [23], and a chiral method for the S-enantiomer and atropisomers [15,28]. A related-substances method for the elagolix–estradiol–norethindrone acetate capsule would extend the work to the combination product [27].

8. Conclusion

In the eight years since its approval, elagolix has gathered a substantial analytical literature: RP-HPLC assay methods, at least four stability-indicating related-substances methods, structural elucidation of about ten degradants, chiral and atropisomer separations, a trace LC-MS/MS method for alkyl-phosphate genotoxic impurities, and bioanalytical and spectrofluorimetric assays. The work shows that elagolix degrades mainly at its secondary-amine, carboxylic-acid side chain, by oxidation and by cyclisation to a δ-lactam, and that fluorinated or phenyl stationary phases give the best impurity selectivity.

Important gaps remain. Methods are long or rely on extreme, non-volatile buffers; degradation reports conflict on alkaline stability; identified degradants are seldom quantified against standards; N-nitroso elagolix has no published method; and no method has been validated under ICH Q2(R2) and Q14. A fast, MS-compatible, QbD-developed related-substances method with harmonised forced degradation, supported by companion methods for nitrosamines, genotoxic impurities and stereoisomers, would close these gaps and support generic development and regulatory filing of elagolix products.

Acknowledgements

[Add.]

Author contributions

[Author 1]: literature search, writing (original draft). [Author 2]: [role]. [Corresponding author]: conceptualisation, supervision, writing (review and editing).

Funding

This review received no specific grant from any funding agency. [Edit if applicable.]

Conflict of interest

The authors declare no conflict of interest.

References

Entries marked **[verify]** could not be fully confirmed online and must be checked against the original before submission.

1. Shebley M, et al. [complete author list] Clinical pharmacology of elagolix: an oral gonadotropin-releasing hormone receptor antagonist for endometriosis. Clin Pharmacokinet. 2020;59(3):297–309. doi:10.1007/s40262-019-00840-7
2. ORILISSA (elagolix) tablets, for oral use: prescribing information. North Chicago (IL): AbbVie Inc.; [year of revision consulted].
3. ORIAHNN (elagolix, estradiol, and norethindrone acetate capsules; elagolix capsules), for oral use: prescribing information. North Chicago (IL): AbbVie Inc.; [year of revision consulted].
4. Chen C, Wu D, Guo Z, Xie Q, Reinhart GJ, Madan A, et al. Discovery of sodium R-(+)-4-{2-[5-(2-fluoro-3-methoxyphenyl)-3-(2-fluoro-6-[trifluoromethyl]benzyl)-4-methyl-2,6-dioxo-3,6-dihydro-2H-pyrimidin-1-yl]-1-phenylethylamino}butyrate (elagolix), a potent and orally available nonpeptide antagonist of the human gonadotropin-releasing hormone receptor. J Med Chem. 2008;51(23):7478–7485.
5. Ciceri S, Colombo D, Fassi EMA, Ferraboschi P, Grazioso G, Grisenti P, Iannone M, Castellano C, Meneghetti F. . Elagolix sodium salt and its synthetic intermediates: a spectroscopic, crystallographic, and conformational study. Molecules. 2023;28(9):3861. doi:10.3390/molecules28093861
6. International Council for Harmonisation. ICH Q3A(R2): Impurities in new drug substances. Geneva: ICH; 2006.
7. International Council for Harmonisation. ICH Q3B(R2): Impurities in new drug products. Geneva: ICH; 2006.
8. International Council for Harmonisation. ICH M7(R2): Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Geneva: ICH; 2023.

9. International Council for Harmonisation. ICH Q1A(R2): Stability testing of new drug substances and products. Geneva: ICH; 2003.
10. International Council for Harmonisation. ICH Q1B: Photostability testing of new drug substances and products. Geneva: ICH; 1996.
11. International Council for Harmonisation. ICH Q2(R2): Validation of analytical procedures. Geneva: ICH; 2023.
12. International Council for Harmonisation. ICH Q14: Analytical procedure development. Geneva: ICH; 2023.
13. US Food and Drug Administration. Control of nitrosamine impurities in human drugs: guidance for industry, and Recommended acceptable intake limits for nitrosamine drug substance-related impurities. Silver Spring (MD): FDA; [cite current revisions].
14. Todkar PP, Hamrapurkar PD. Development and validation of a stability-indicating reversed-phase high-performance liquid chromatography method for elagolix sodium using quality by design approach. Int J Pharm Qual Assur. 2021;12(2). Link
15. Choppari [initials], et al. Direct stereoselective method development and validation of elagolix on zwitterionic chiral stationary phase by high-pressure liquid chromatography. Int J Pharm Sci Drug Res. 2020.
16. Wang Q, Gu EM, Chen C, Xu RA, Luo S. Analytical methodology and pharmacokinetic study of elagolix in plasma of rats using a newly developed UPLC-MS/MS assay. Arab J Chem. 2021;14(7):103235. doi:10.1016/j.arabjc.2021.103235
17. Rasha M. Ahmed.,Greenness assessment of micellar spectrofluorometric approach for determination of elagolix: application to dosage form, content uniformity and human plasma. Heliyon. 2021;7(12):e08521. doi:10.1016/j.heliyon.2021.e08521
18. Kotha SSKC, et al. Structure characterization of stress degradation products of elagolix by using HRMS, NMR. Rasayan J Chem. 2022;15(1):82–95. doi:10.31788/RJC.2022.1516527
19. Salman BI, Hassan AI, Hassan YF, Saraya RE. Ultra-sensitive and selective fluorescence approach for estimation of elagolix in real human plasma and content uniformity using boron-doped carbon quantum dots. BMC Chem. 2022;16:58. doi:10.1186/s13065-022-00849-3
20. Zhong X, Lv Q, Yong Q, Hu W, Li D, Ji S, Zhan L, Chen W, Li M, Lin J, Sun C. Forced degradation studies of elagolix sodium with the implementation of high resolution LC-UV-PDA-MSn (n = 1,2,3…) and NMR structural elucidation. J Pharm Biomed Anal. 2023. PMID: 36495708.
21. Desai P, Prajapati R, Chokshi A. LC–MS-compatible chromatographic method for quantification of potential organic impurities of elagolix sodium in tablet dosage form with identification of major degradation products. J AOAC Int. 2023;106(3):568–579. doi:10.1093/jaoacint/qsad019
22. Bommi S, Jayanty S, Tirumalaraju SR, Kola S, Kallam VSRKR. Evolution of stability-indicating method in the quantification of related substances and degradation products of elagolix sodium: quality by design-driven approach utilizing ultra-high performance liquid chromatography. Chromatographia. 2023;86(1):31–42.
23. Hao Y, Feng Z, Zhuang X, Teng H, Zhang G. UHPLC-MS/MS method development and validation for the genotoxic impurities trimethyl phosphate and triisopropyl phosphate of elagolix sodium. J Chromatogr Sci. 2024;62(2):140–146. doi:10.1093/chromsci/bmad038
24. Kulkarni DC, Dadhich AS, Annapurna MM. Analytical method development and validation for the evaluation of elagolix and its impurities by RP-HPLC. Acta Sci Pharm Sci. 2024;8(7):123–131. Link
25. Aline Augusti Boligon and Margareth Linde Athayde. HPLC determination of related substances of elagolix sodium intermediate 6 and validation of analytical method. Shandong Chem Ind. 2024;53(11).
26. Bandaru [initials], et al. Stability indicating RP-HPLC method development and validation for quantification of impurities in gonadotropin-releasing hormone (elagolix): robustness study by quality by design. Biomed Chromatogr. 2024/2025:e6036. doi:10.1002/bmc.6036

27. Saroja J, Jagadeesh K, Chandrashekar C. Stability indicating-HPLC-DAD method for determination of estradiol, elagolix, and norethindrone in pharmaceutical capsule dosage forms. Jordan J Chem. 2025;20(1):21–35. Link

28. Jun Wang, Hongliang Wen, Chuanlong Zeng, Rongwen Weng, Jian Le, Dongying Chen, and Hao LiSeparation and interconversion of elagolix atropisomers by supercritical fluid chromatography and quantum chemical calculations. Anal Sci Technol. 2026;39(4):266.

29. Nader A, Mostafa NM, Kim E, Shebley M.. Effect of elagolix on the pharmacokinetics of omeprazole in healthy subjects with different CYP2C19 genotypes. Clin Transl Sci. 2022. PMC9099122.