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LC-MS/MS Method Development and Validation for Quantification of Lenvatinib in K2EDTA Human Plasma: A Comprehensive Review

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Abstract: Lenvatinib is an oral multi-targeted tyrosine kinase inhibitor of VEGFR1–3, FGFR1–4, PDGFRα, RET and KIT, used in radioiodine-refractory differentiated thyroid cancer, hepatocellular carcinoma, renal cell carcinoma and endometrial carcinoma. Its pharmacokinetics vary widely between patients, more than 60% of patients need dose reduction or interruption, and exploratory trough targets of about 40–90 ng/mL have been proposed. These features have driven the development of LC-MS/MS methods for plasma quantification in support of clinical pharmacology and therapeutic drug monitoring (TDM).

This review surveys the bioanalytical methods published for lenvatinib between 2012 and 2026. It covers single-analyte LC-MS/MS methods in plasma and serum, the sponsor's multi-laboratory validation and cross-validation, multi-analyte TKI panels, methods for total and unbound drug, microsampling (dried blood spots), HPLC-UV and preclinical methods. For each, sample volume, anticoagulant, extraction, internal standard, chromatography, MS transitions, calibration range and validation findings are summarised.

The literature shows that protein precipitation with a stable-isotope-labelled internal standard and a short C18 gradient can meet TDM needs over 0.5–2000 ng/mL. However, most methods used sodium-heparin plasma, only one plasma method reports K_2EDTA, several calibration ranges do not bracket the clinical trough window, matrix effects are often large, and no single-analyte method has been validated under ICH M10. A strategy for a K_2EDTA human plasma method aligned with ICH M10 is proposed.

Keywords: Lenvatinib; LC-MS/MS; K_2EDTA plasma; bioanalytical method validation; ICH M10; therapeutic drug monitoring; tyrosine kinase inhibitor

1. Introduction

Lenvatinib (E7080, Lenvima®) is an oral multi-kinase inhibitor that blocks vascular endothelial growth factor receptors 1–3, fibroblast growth factor receptors 1–4, PDGFRα, RET and KIT [1]. It is approved for radioiodine-refractory differentiated thyroid cancer (DTC) at 24 mg/day, unresectable hepatocellular carcinoma (HCC) at 8 or 12 mg/day by body weight, advanced renal cell carcinoma with everolimus or pembrolizumab, and advanced endometrial carcinoma with pembrolizumab at 20 mg/day [1–5].

Toxicity limits its use. Hypertension, fatigue, proteinuria, diarrhoea and hand–foot skin reaction are common, and most patients in pivotal and real-world studies needed dose reductions or interruptions [2,3]. Exposure varies widely between patients at the same dose because of differences in body weight, hepatic function, CYP3A4 and aldehyde oxidase activity, and drug interactions [1,27]. Exposure–response studies have linked trough concentration (Cmin) with response and toxicity. Proposed thresholds include a Cmin above 42.68 ng/mL for better response in HCC [7], an exploratory target of 51.5 ng/mL [6], a

toxicity threshold of 88 ng/mL in Japanese DTC patients [8], and a target range of about 37–71 ng/mL in HCC [9]. These findings make lenvatinib a candidate for therapeutic drug monitoring (TDM).

TDM, bioequivalence and clinical pharmacology studies all depend on a validated bioanalytical method. LC-MS/MS is the method of choice because it offers selectivity, sensitivity and short run times. Regulators expect such methods to be validated in the same matrix and with the same anticoagulant as the study samples. ICH M10, adopted in 2022, harmonised these expectations and treats a change of anticoagulant as a change that needs at least partial validation [28].

K_2EDTA is the anticoagulant most often used for clinical pharmacokinetic sampling and for routine hospital blood collection. Yet, as this review shows, most published lenvatinib methods were validated in sodium-heparin plasma. This review collates the published bioanalytical methods for lenvatinib, summarises their conditions, findings and limitations, identifies the gaps, and sets out the scope for developing and validating an LC-MS/MS method for lenvatinib in K_2EDTA human plasma under ICH M10.

2. Drug profile and pharmacokinetics relevant to bioanalysis

Lenvatinib's high protein binding, wide exposure range and simple MS fragmentation shape every design choice in its bioanalysis. Table 1 summarises the properties that matter.

Table 1. Physicochemical and pharmacokinetic profile of lenvatinib relevant to LC-MS/MS

Property	Value	Bioanalytical implication
Chemical name	4-[3-Chloro-4-(N′-cyclopropylureido)phenoxy]-7-methoxyquinoline-6-carboxamide	Basic quinoline nitrogen ionises well in positive ESI
Formula and mass	$C_{21}H_{19}ClN_4O_4$; 426.9 g/mol (free base); marketed as mesylate (523.0 g/mol)	Prepare stocks as free-base equivalents; account for salt factor
MS transition	$[M+H]^+$ m/z 427 → 370 (quantifier); 312 and 344 as qualifiers [12,20,24]	Single dominant fragment; chlorine isotope pattern aids confirmation
Stable-isotope IS	Lenvatinib-d4, -d5 and $^{13}C_6$-lenvatinib reported [13,14,20,24]	Commercially available; preferred for matrix-effect compensation
Absorption	Tmax about 1–4 h [1]	Trough sampling just before the next dose is preferred for TDM
Half-life	About 28 h [1]	Steady state reached within about a week; daily dosing gives a modest peak–trough ratio
Protein binding	97.5–99%, mainly to albumin [1,26]	Requires efficient protein disruption; unbound fraction needs dialysis or ultrafiltration [11]
Metabolism	CYP3A4 and aldehyde oxidase; metabolites include decyclopropylated, O-desmethyl, N-oxide and O-dearylated products [10,27]	Metabolites must not interfere with the analyte channel; N-oxide may revert in the source
Elimination	Mainly faecal (about 64%), with about 25% in urine [1]	Renal impairment has little effect on total exposure
Clinical concentrations	Reported Cmin of about 10–100 ng/mL and Cmax up to several hundred ng/mL at approved doses [6,20]	Calibration should span about 1–1000 ng/mL, with dilution integrity above ULOQ

Two properties need particular care. First, binding of 97.5–99% means total-drug methods must break protein binding completely, while free-drug measurement requires equilibrium dialysis or ultrafiltration and a very low LLOQ [11,26]. Second, the N-oxide and other oxidative metabolites share the core structure, so they can produce in-source fragments or isobaric signals unless they are separated chromatographically from lenvatinib [10].

3. Regulatory framework for bioanalytical validation

ICH M10 (2022) is now the single reference for chromatographic bioanalytical validation in the ICH regions and replaces the earlier FDA (2018), EMA (2011) and Japanese MHLW (2013) guidance, under which all lenvatinib methods before 2023 were validated [28–31]. Its main requirements for an LC-MS/MS method in K_2EDTA plasma are summarised in Table 2.

Table 2. ICH M10 validation requirements applied to a lenvatinib K_2EDTA plasma method [28]

Parameter	Requirement	Note for lenvatinib
Matrix and anticoagulant	Validate in the same matrix and anticoagulant as study samples; a change of anticoagulant needs partial validation	Heparin-validated methods cannot be used for K_2EDTA samples without at least partial validation
Selectivity	At least 6 individual sources; interference ≤ 20% of LLOQ response (analyte) and ≤ 5% (IS); include haemolysed and lipaemic plasma	Patients with HCC often have hyperbilirubinaemia; icteric plasma is worth testing
Calibration curve	At least 6 non-zero levels; ≥ 75% within ±15% (±20% at LLOQ)	Range should bracket the 37–88 ng/mL trough window with margin
Accuracy and precision	LLOQ, low, medium and high QC; within-run and between-run; bias ±15% and CV ≤ 15% (±20% and ≤ 20% at LLOQ)	Three runs over at least two days
Matrix effect	At least 6 lots at low and high QC; accuracy ±15%, CV ≤ 15%	IS-normalised matrix factor with a stable-isotope IS
Carry-over	Blank after ULOQ ≤ 20% of LLOQ and ≤ 5% of IS	Lenvatinib's hydrophobic urea can adsorb to injector surfaces
Dilution integrity	Accuracy and precision within ±15% after dilution	Needed for peak samples above ULOQ
Stability	Stock and working solutions; bench-top; ≥ 3 freeze–thaw cycles; long-term frozen; processed-sample (autosampler); whole blood before centrifugation	Whole-blood stability in K_2EDTA tubes is not reported in the lenvatinib literature
Incurred sample reanalysis	10% of the first 1000 samples and 5% thereafter; ≥ two-thirds within ±20% of the mean	Required for PK and BE studies
Cross-validation	When data from different methods or laboratories are combined	Demonstrated for lenvatinib across five laboratories [14]

For TDM in hospital laboratories, the IATDMCT and clinical laboratory standards apply the same core principles, though not all academic methods follow the full M10 programme [22]. A method intended both for clinical studies and TDM should therefore meet M10 in full.

4. Review of published bioanalytical methods

About 15 bioanalytical reports for lenvatinib have appeared since 2012. They fall into five groups: sponsor methods supporting clinical development (2012–2018), academic single-analyte LC-MS/MS methods for plasma (2017–2026), multi-analyte TKI panels for TDM [16,17,19,23], microsampling and non-MS methods, and preclinical methods in rat plasma. Table 3 summarises each in chronological order.

Table 3. Published bioanalytical methods for lenvatinib

#	Author, year [ref]	Matrix and volume	Extraction and IS	Chromatography and MS	Range (ng/mL)	Key findings	Observations and limitations
1	Dubbelman et al., 2012 [10]	Plasma 250 µL; also urine, faeces, whole blood	PP with acetonitrile, evaporation and reconstitution; IS ER-227326 (analogue)	XTerra MS C18, 50 × 2.1 mm; formic acid–acetonitrile gradient, 0.2 mL/min; API 3000; run about 21 min	0.25–50 (plasma, with 4 metabolites); 0.25–500 (whole blood)	$r^2 \geq 0.994$; LLOQ bias ±20%, CV < 20%; cross-analyte interference addressed; used in mass balance study	First method. ULOQ below the TDM target Cmin; long run; analogue IS
2	Mano and Kusano, 2015 [11]	Serum 0.8 mL dialysed 18 h at 37 °C; PBS dialysate	Organic-solvent extraction	Symmetry Shield RP8; 2 mM ammonium acetate pH 4.0–acetonitrile (3:2), isocratic, 0.2 mL/min; API 4000	0.08–400 (serum); 0.04–16 (PBS)	Total and unbound drug; stable on bench, freeze–thaw, processed and frozen; confirmed high protein binding	Specialised protein-binding assay; serum, not plasma
3	Ogawa-Morita et al., 2017 [12]	Sodium-heparin plasma 250 µL	PP with acetonitrile, N_2 drying, reconstitution; IS propranolol	XTerra MS C18, 50 × 2.1 mm; 0.1% formic acid–acetonitrile gradient, 0.2 mL/min; 15 min; m/z 427.6 → 371.0	9.6–200	Accuracy 95.8–108.3%; CV < 6.7%; matrix effect +12.9 to +15.7%; Japanese MHLW guideline	Analogue IS; recovery only 66.8%; narrow range; 10–14% loss after three freeze–thaw cycles
4	Srikanth and Prameela Rani, 2017 [13]	Sodium-heparin plasma 200 µL	LLE; IS lenvatinib-d4	RP-LC-MS/MS; 8 min	0.0102–0.5016 (10.2–501.6 pg/mL)	$r^2 \geq 0.995$; proposed for PK and bioavailability studies	Range about 100-fold below clinical concentrati

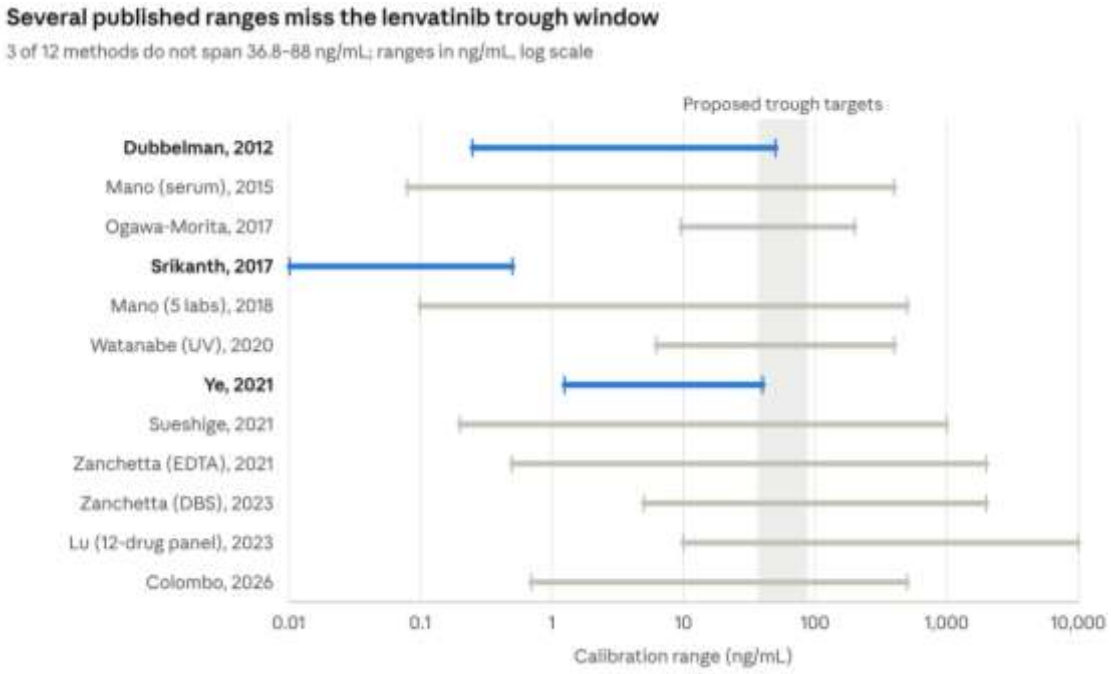
							ons; patient samples would need large dilutions
5	Mano, 2018 [14]	Sodium-heparin plasma 50–200 µL, seven methods at five laboratories	PP (acetonitrile–methanol), LLE (diethyl ether or MTBE–isopropanol) or SPE (HLB, MCX); IS ER-227326 or $^{13}C_6$-lenvatinib	Symmetry Shield RP8, Hypersil Gold, Synergi Polar-RP, Luna C18(2); 0.2–0.45 mL/min	0.1–100 to 0.25–500	All methods met guideline criteria; cross-validation QC accuracy within ±15.3%, clinical-sample bias within ±11.6%	Shows that diverse methods give comparable data. All heparin plasma; pre-M10; ULOQs of 100–500 ng/mL
6	Watanabe et al., 2020 [18]	Serum	SPE (ODS cartridge); IS	ODS column; 0.02 M phosphate pH 6.7–acetonitrile (50:50); 1.0 mL/min; UV 244 nm	6.25–400	r = 0.9998; recovery ≥ 97%; CV < 4.7% intra-day, < 6.0% inter-day; 5 patients	HPLC-UV alternative where LC-MS/MS is unavailable; LLOQ near lowest troughs
7	Aghai et al., 2021 [16]	Serum and plasma	PP; stable-isotope IS for each of 10 kinase inhibitors	LC-MS/MS; 7.0 min acquisition	**[verify lenvatinib range]**	Validated to FDA and EMA guidance; applied in routine TDM	Panel method; lenvatinib-specific performance not detailed in abstract
8	Ye et al., 2021 [19]	Human plasma	PP	UPLC C18; water–acetonitrile gradient; 3.5 min	1.25–40 (lenvatinib)	First simultaneous sorafenib–lenvatinib–apatinib assay; stability in plasma and whole blood assessed	ULOQ below the 51.5 ng/mL target; many troughs would need dilution
9	Sueshige et al. [15]	Plasma 100 µL	SPE; IS lenvatinib-d4	LC-MS/MS; 4–6 min	0.2–1000	Range suitable for TDM; short	SPE adds cost and time

						run	
10	Zanchetta et al., 2021 [20]	K-EDTA plasma 100 µL	PP with 500 µL cold methanol + 0.1% formic acid; IS lenvatinib-d4	Synergi Fusion-RP C18, 30 × 2 mm, 4 µm, 50 °C; water/methanol–isopropanol with 0.1% formic acid; 0.6 mL/min; 4 min; API 4000	0.50–2000	CV ≤ 11.3%; accuracy 96.3–109%; recovery ≥ 95.6%; IS-normalised matrix factor CV ≤ 2.8%; stable 315 days at −80 °C; dilution integrity; ISR on 14 samples; applied to HCC patients	Only EDTA plasma method found. Strong ion enhancement (136–157%); quadratic fit; carry-over and whole-blood stability not reported
11	Talari et al., 2022 [21]	Rat plasma	IS carfilzomib	XBridge phenyl, 150 × 4.6 mm, 3.5 µm; 0.1% formic acid–acetonitrile gradient, 1 mL/min; 30 min	10–200% of target	Lenvatinib and related impurities, including an active metabolite, separated	Preclinical; long run
12	Zanchetta et al., 2023 [22]	Venous DBS, 10 µL volumetric spot	Whole-spot extraction	LC-MS/MS; 4 min	5.00–2000	Two filter papers; haematocrit effect CV ≤ 6.3%; CV ≤ 8.8%; R^2 ≥ 0.996 against plasma	Enables home sampling; needs plasma-conversion model
13	Lu et al., 2023 [23]	Human plasma	PP	UPLC-MS/MS (QTRAP 4500)	10–10 000	12-drug panel for HCC chemotherapy, TKIs and analgesics	LLOQ of 10 ng/mL above some reported troughs
14	Colombo et al., 2026 [24]	Sodium-heparin plasma 50 µL	LLE (cold acetonitrile, then 1-chlorobutane); IS lenvatinib-d5	Luna Omega Polar C18, 100 × 2.1 mm, 1.6 µm; 0.1% formic acid–methanol gradient, 0.5	0.7–500	Recovery > 95%; matrix enhancement 6–16%; dose-proportional concentrations in 19 DTC patients; inter-patient variability 17–52%	Heparin plasma; LLE more laborious than PP

				mL/min; about 10 min; QTRAP 5500; m/z 427 → 370			
15	Huhn et al., 2026 [25]	Human plasma	Minimal preparation	LC-MS/MS	—	Belzutifan, cabozantinib, cabozantinib N-oxide and lenvatinib; validated to ICH M10	Conference poster; full data not yet published

5. Critical observations

5.1 Calibration ranges often fail to match clinical concentrations. Reported troughs run from about 9 to 100 ng/mL, and peaks reach several hundred ng/mL [6,20]. Three of the 12 human methods with stated ranges do not span the proposed 37–88 ng/mL trough window, either because the ULOQ is too low [10,19] or because the whole range is in the picogram range [13]. Others have LLOQs of 6–10 ng/mL that would miss the lowest troughs seen at reduced doses [12,18,23].



Ranges from Table 3 [10–24]; trough window from Noda, Hata, Verheijen and Nagahama [6–9]

The best-fitted ranges for TDM are 0.2–1000 ng/mL [15], 0.50–2000 ng/mL [20] and 0.7–500 ng/mL [24], combined with validated dilution for higher samples.

5.2 Heparin plasma dominates; K_2EDTA is barely represented. The sponsor's seven methods [14], Ogawa-Morita et al. [12], Srikanth et al. [13] and Colombo et al. [24] all used sodium-heparin plasma. Only Zanchetta et al. used EDTA plasma, without specifying the K_2 or K_3 salt [20]. No study has compared lenvatinib recovery or matrix effect between heparin and EDTA plasma, or reported whole-blood stability in EDTA tubes.

5.3 Protein precipitation with a stable-isotope IS is sufficient. PP, LLE and SPE all gave acceptable data in the cross-validation [14]. Recent PP methods with lenvatinib-d4 achieved recoveries of 95–102% from 100 µL of plasma in a 4 min run [20]. LLE gave cleaner extracts with smaller matrix effects (6–16%) at the cost of extra steps [24]. Analogue IS such as propranolol or ER-227326 compensate less well for matrix effects and extraction losses; the propranolol method recovered only 66.8% [12].

5.4 Matrix effects can be large and are not always reported consistently. Zanchetta et al. found ion enhancement of 136–157% that the deuterated IS corrected (IS-normalised CV ≤ 2.8%) [20]. Ogawa-Morita et al. reported a matrix effect of about +15% but described it in one place as extraction efficiency [12]. Short ballistic gradients on 30–50 mm columns elute lenvatinib close to phospholipids; monitoring phospholipid transitions and using longer retention or LLE reduces the risk.

5.5 Chromatography is converging on short C18 gradients with formic acid. Most methods use C18 or polar-embedded C18 columns, 0.1–0.2% formic acid in water, and methanol or acetonitrile [12,14,20,24]. Run times have fallen from 15–21 min [10,12] to 4–7 min [16,20,22].

5.6 Stability is well covered except for whole blood. Lenvatinib is stable for 315 days at −80 °C, through three freeze–thaw cycles, for 4–24 h on the bench and for up to 94 h in the autosampler [12,20]. One method reported 10–14% loss after three freeze–thaw cycles at −20 °C [12]. Whole-blood stability before centrifugation, which matters for hospital sampling, has been assessed only in a multi-analyte method [19].

5.7 Validation predates ICH M10. All single-analyte plasma methods were validated to FDA, EMA or Japanese guidance [12–14,20,24]. Only a 2026 multi-analyte poster claims ICH M10 compliance [25]. Carry-over, haemolysed and lipaemic plasma, and incurred-sample reanalysis are reported inconsistently.

5.8 Microsampling is emerging. Volumetric DBS validated over 5–2000 ng/mL correlated well with plasma ($R^2 \geq 0.996$) [22]. These approaches still rely on a validated plasma reference method for conversion.

6. Gaps identified

No published method provides a single-analyte LC-MS/MS assay for lenvatinib validated specifically in K_2EDTA human plasma under ICH M10, with a range fitted to clinical troughs and peaks. Table 4 sets out the gaps.

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

#	Gap	Evidence	Improvement needed
1	Almost no K_2EDTA-validated methods	Heparin plasma in 10 of 11 plasma methods with stated anticoagulant [12–14,24]; EDTA salt unspecified in [20]	Full validation in K_2EDTA plasma, with partial validation or bridging to heparin data if needed
2	No ICH M10 validation for a single-analyte method	Pre-2022 guidance in all single-analyte methods [12–14,20,24]	M10-compliant programme, including haemolysed and lipaemic plasma, carry-over and ISR
3	Calibration ranges mismatched to clinical concentrations	Three of 12 ranges miss the 37–88 ng/mL window; some LLOQs of 6–10 ng/mL [10,12,13,18,19,23]	About 0.5–1000 ng/mL, with validated 10-fold dilution
4	Whole-blood stability in EDTA tubes not reported	Assessed only in a multi-analyte method [19]	Stability at room temperature and on ice for up to 2–4 h before centrifugation
5	Large or inconsistently reported matrix effects	Enhancement of 136–157% [20]; ambiguous reporting [12]	Matrix factor in at least 6 K_2EDTA lots, including haemolysed, lipaemic and icteric plasma; phospholipid monitoring
6	Analogue internal standards in some methods	Propranolol and ER-227326 [12,14]	Stable-isotope IS (lenvatinib-d4 or $^{13}C_6$)
7	Carry-over rarely reported	Not reported in [20]; inconsistently elsewhere	Carry-over test after ULOQ in every validation run
8	Metabolite interference not examined in recent methods	Metabolites characterised only in [10]	Chromatographic separation of the N-oxide and other metabolites; check for in-source conversion

9	Long or laborious sample preparation	LLE, SPE or evaporation steps [10,12,15,24]	Single-step PP in 96-well format with ≤ 100 µL plasma
10	Limited application data in Indian and other non-Japanese, non-European populations	Patient data mainly from Japan and Italy [7,8,20,24]	Application to local patient trough samples and BE study support
11	No greenness or cost assessment	None reported	Low solvent volumes, short runs and AGREE or BAGI scoring

7. Scope and proposed strategy for the new K_2EDTA plasma method

The literature supports a straightforward design: protein precipitation, a stable-isotope IS and a short C18 gradient [14,20], applied to K_2EDTA plasma over a range fitted to clinical concentrations and validated in full to ICH M10 [28].

7.1 Method objective

Quantify total lenvatinib in K_2EDTA human plasma from ≤ 100 µL, over about 0.5–1000 ng/mL with validated 10-fold dilution, in a run of ≤ 5 min, meeting ICH M10 criteria, for use in pharmacokinetic, bioequivalence and TDM studies.

7.2 Proposed starting conditions

Table 5. Suggested initial LC-MS/MS conditions, to be optimised

Parameter	Proposed starting point	Rationale
Matrix	K_2EDTA human plasma, 50–100 µL	Matches clinical sampling; low volume [20,24]
Internal standard	Lenvatinib-d4 (or $^{13}C_6$-lenvatinib) at about mid-range concentration	Corrects the large ion enhancement seen with short gradients [14,20]
Extraction	PP with 3–5 volumes of acetonitrile (or methanol) containing 0.1% formic acid; vortex, centrifuge, dilute supernatant; optional phospholipid-removal plate	Recoveries of 95–102% reported for PP [20]; plate reduces matrix effect
Column	C18 or polar-embedded C18, 50 × 2.1 mm, 1.7–2.7 µm, 40 °C	Used in most published methods [12,14,24]
Mobile phase	A: 0.1% formic acid (optionally with 2 mM ammonium formate) in water; B: acetonitrile or methanol with 0.1% formic acid	Formic acid gives strong $[M+H]^+$; ammonium formate can sharpen peaks
Gradient	About 20% to 90% B over 2.5 min, wash and re-equilibrate; total ≤ 5 min; retention factor ≥ 3	Keeps lenvatinib clear of early-eluting phospholipids
Ionisation and MRM	ESI+; lenvatinib m/z 427.1 → 370.1 (quantifier) and 427.1 → 312.1 (qualifier); IS transition per its label position, e.g. 431 → 370 for d4	Transitions reported in [12,20,24]
Calibration	8 non-zero levels, 0.5–1000 ng/mL; linear, $1/x^2$ weighting	Covers troughs of about 10–100 ng/mL and most peaks
QC levels	LLOQ 0.5, low 1.5, medium about 400, high about 800 ng/mL; dilution QC about 5000 ng/mL diluted 10-fold	Meets M10 placement rules

7.3 Development and validation workflow

1. Optimise MS parameters by infusion of lenvatinib and IS; confirm the chlorine isotope pattern and choose quantifier and qualifier transitions.
2. Screen two columns and two organic modifiers for peak shape, retention and separation from metabolites, especially the N-oxide.
3. Compare PP solvents and PP with phospholipid removal for recovery, matrix factor and phospholipid breakthrough.

4. Test carry-over after ULOQ and adjust needle wash (for example acetonitrile–isopropanol–water with formic acid).
5. Run full ICH M10 validation in K_2EDTA plasma (Table 6).
6. Apply to trough and peak samples from patients receiving lenvatinib, with incurred-sample reanalysis; cross-validate against a heparin-plasma method if earlier data must be combined.

7.4 Validation plan

Table 6. Proposed ICH M10 validation for lenvatinib in K_2EDTA plasma

Parameter	Design	Acceptance criterion
Selectivity	6 individual K_2EDTA lots plus haemolysed (2%) and lipaemic plasma; consider icteric plasma for HCC patients	Interference ≤ 20% of LLOQ (analyte), ≤ 5% (IS)
Calibration curve	8 levels in each run	≥ 75% of standards within ±15% (±20% at LLOQ)
Accuracy and precision	LLOQ, low, medium, high QC; 5 replicates; 3 runs over ≥ 2 days	Bias ±15%, CV ≤ 15% (±20%, ≤ 20% at LLOQ)
Matrix effect	Low and high QC in 6 lots, including haemolysed and lipaemic	IS-normalised accuracy ±15%, CV ≤ 15%
Recovery	Low, medium and high QC versus post-extraction spikes	Consistent and reproducible
Carry-over	Blank after ULOQ in each run	≤ 20% of LLOQ, ≤ 5% of IS
Dilution integrity	Dilution QC above ULOQ diluted 10-fold with blank K_2EDTA plasma	Bias ±15%, CV ≤ 15%
Stability	Stock and working solutions; whole blood (room temperature and on ice, 2–4 h); bench-top; 3–5 freeze–thaw cycles at −20 and −80 °C; long-term frozen; processed samples in autosampler	Within ±15% of nominal
Incurred sample reanalysis	10% of study samples	≥ two-thirds within ±20%

Under ICH M10, a change of anticoagulant counter-ion (for example K_2 to K_3 EDTA) is not usually treated as a change of matrix, but a switch between heparin and EDTA is [28]. Validating directly in K_2EDTA therefore makes the method usable for most clinical and hospital samples without further bridging.

8. Conclusion

Since 2012, about 15 bioanalytical methods for lenvatinib have been reported, from the sponsor's multi-laboratory LC-MS/MS assays to academic TDM methods, multi-analyte panels and dried-blood-spot assays. Together they show that lenvatinib is easily ionised (m/z 427 → 370), stable in frozen plasma, and quantifiable from 50–100 µL of plasma after simple protein precipitation with a stable-isotope internal standard in runs of 4–10 min.

The main gaps are the near absence of K_2EDTA-validated methods, the lack of ICH M10 validation for any single-analyte assay, calibration ranges that often miss the 37–88 ng/mL trough window, unreported whole-blood stability, and large or inconsistently reported matrix effects. A protein-precipitation LC-MS/MS method with lenvatinib-d4, a range of about 0.5–1000 ng/mL and full ICH M10 validation in K_2EDTA plasma would close these gaps and support pharmacokinetic, bioequivalence and TDM studies of lenvatinib.

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