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# STABILITY-INDICATING UPLC METHOD FOR RIVAROXABAN AND ITS DEGRADATION PRODUCTS

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**Abstract**

Rivaroxaban is a widely prescribed oral factor Xa inhibitor, and regulators expect its assay and impurity methods to separate the intact drug from anything that forms on storage. This review brings together published work on stability-indicating ultra-performance liquid chromatography (UPLC) for rivaroxaban and its degradation products. It covers the chemistry that makes the molecule vulnerable, the design of forced degradation studies under ICH guidance, the choices that shape a sub-2 µm gradient separation, and the validation and system suitability evidence that supports routine use. Hydrolytic stress is repeatedly described as the main source of degradants, whereas peak purity and mass balance are reported less consistently. The article is a narrative review of published literature and reports no new experimental data.

## 1. INTRODUCTION

Rivaroxaban entered clinical use as the first oral direct factor Xa inhibitor, and its adoption was quick. Large trials showed that fixed oral doses could prevent venous thromboembolism after hip surgery and could treat established venous thromboembolism without routine coagulation monitoring [1, 2]. Each batch has to remain within specification until the end of shelf life. That requirement lands on the analyst. A method that reports only the parent peak can look perfectly healthy while the product quietly loses potency or accumulates impurities that nobody is measuring. For an anticoagulant with a narrow margin between benefit and bleeding, an unreliable stability assay is not a

minor inconvenience [3, 4]. Its direct inhibition of factor Xa, free and bound, is what makes fixed dosing possible [5]. Pharmacopoeial monographs exist for the drug and its tablets, but the developer of a new product or a new manufacturing site still has to show that the in-house procedure is fit for its stated purpose.

The regulatory vocabulary for this problem is well settled. ICH Q1A(R2), and the WHO guidance built on the same principles, ask for stress testing so that likely degradation products and pathways are known, and it expects the analytical procedures used in stability studies to be stability-indicating and validated [6]. Companion ICH guidelines (Q3A and Q3B) set reporting, identification and qualification thresholds for impurities in drug substances and products, and specialist reviews describe how impurities are identified and qualified [7, 8]. What the guidelines leave open is how an individual laboratory should prove that its method is stability-indicating. The literature fills that gap with a recognisable workflow: stress the drug under defined conditions, develop a separation that resolves every product formed, show that the main peak is spectrally pure, and then validate the procedure [9, 10, 11]. This review follows that sequence for rivaroxaban. None of these documents prescribes a column, a mobile phase or a gradient. They define what must be shown and leave the how to the analyst, which goes a long way towards explaining the variety of methods in print.

Why UPLC rather than conventional high-performance liquid chromatography? The short answer is time and resolution. Columns packed with particles below 2 µm, run at pressures beyond 600 bar, give narrower peaks and higher efficiency per unit length, which usually means shorter runs and better detection of small impurity peaks next to a large parent peak [12, 13]. For a drug that may generate several closely related degradants, that extra resolving power is attractive. It also changes the practical demands on the instrument, with smaller dwell volumes, faster detector sampling and tighter limits on extra-column dispersion, and these demands are discussed later in the context of method transfer.

## 2. CHEMISTRY AND STABILITY PROFILE

Rivaroxaban is a neutral oxazolidinone derivative carrying a chlorothiophene carboxamide and a morpholinone ring, built around a single (S) stereocentre [14]. Its molecular weight is close to 436, it has no strongly ionisable group in the usual chromatographic pH range, and it is poorly soluble in water. Those features matter before any column is chosen. A neutral analyte of moderate lipophilicity is retained mainly by hydrophobic interaction, so mobile phase pH has little effect on its retention but a large effect on any acidic or basic degradant that forms. That asymmetry between parent and products is one reason pH screening remains part of development even for a neutral drug [15, 16]. The tablet strengths are small, from 2.5 mg to 20 mg, so the active ingredient is a minor part of the dosage unit and excipients dominate the sample matrix [4].

The same structure tells an analyst where trouble is likely to start. Three amide-type linkages sit in the molecule: the thiophene carboxamide, the oxazolidinone carbamate and the lactam of the morpholinone ring. Amide and carbamate bonds are the classic targets of acid and base hydrolysis, and general reviews of stress testing list hydrolysis as the dominant route for drugs of this type [10, 17]. Cleavage of the carboxamide would be expected to release a chlorothiophene carboxylic acid and an amine-bearing fragment, both of which are also known as process-related materials in the synthesis of the drug [7, 14]. Distinguishing a degradant from a carried-over intermediate is therefore a genuine analytical question, not a formality. Because the thiophene ring carries chlorine, an oxidative or photochemical route could in principle dechlorinate or hydroxylate it, so that possibility is checked rather than ignored.

Clinical pharmacology adds context for why purity matters at the dose level. Rivaroxaban is well absorbed, and the 10 mg and 15 mg or 20 mg tablets show food-dependent bioavailability, with the higher strengths taken with a meal [4, 18]. Elimination is split

between renal excretion of unchanged drug and metabolism, largely by CYP3A4, CYP2J2 and CYP-independent hydrolysis, and the metabolites are mainly oxidative or hydrolytic [19]. Metabolic hydrolysis products overlap in structure with some chemical degradants, which means that metabolite reference data can help to anticipate peaks seen in stressed samples. They also remind the reader that a degradant is not automatically toxic or inactive, and qualification decisions belong to the impurity thresholds rather than to assumption [8]. Early multiple-dose work in healthy men found predictable, dose-proportional exposure with little accumulation [20], and co-administration studies with drugs that share its elimination routes show exposure changes of varying size [21, 22]. All of this assumes the tablet delivers exactly what the label says.

## 3. FORCED DEGRADATION STRATEGY

Forced degradation is the experiment that makes a method defensible. The aim is not to destroy the drug but to generate a realistic set of degradants, usually targeting a loss of roughly 5 to 20 per cent of the parent, so that the separation is challenged without producing secondary and tertiary fragments that would never appear on a shelf [23, 24]. Typical conditions are acid and base hydrolysis at several concentrations and temperatures, neutral hydrolysis in water, oxidation with hydrogen peroxide, dry heat, humidity, and light exposure. Published guidance recommends beginning mildly and escalating, and it warns against reporting a method as stability-indicating on the strength of a single severe condition [24, 25]. Duration matters as well. A sample left for days under heat may reveal more about the glassware and solvent than about the product, so time points are normally taken early and often.

Reported behaviour of rivaroxaban follows the chemistry reasonably well. The stress studies in the literature describe appreciable degradation under alkaline and acidic hydrolysis, with base usually the more aggressive of the two, and relatively limited change under oxidative, thermal and photolytic conditions, so that the drug is generally regarded as hydrolytically labile but otherwise reasonably robust [10, 17]. The number of degradation products reported varies between laboratories. Part of that spread reflects different stress intensities and part reflects different chromatography, since a peak that is one band on a 15 minute gradient may split into two on a better column. Direct comparison between papers should therefore be cautious, and table 1 collects the main patterns qualitatively. Where a paper reports no degradation under a given condition, it is worth checking that the exposure was long and strong enough to count as a real test. The entries in table 1 divide, as fig. 1 shows, between stress, chromatography and validation topics.

**Table 1: Summary of reported stress behaviour, chromatographic features and validation points for rivaroxaban**

| Item | Reported finding or typical feature | Ref. |
|---|---|---|
| Acid hydrolysis dilute mineral acid, heat | Appreciable degradation attributed to cleavage of amide-type bonds; several products resolved | [10, 17] |
| Base hydrolysis dilute alkali | Usually the most aggressive condition; extensive loss of parent and multiple degradant peaks | [17] |
| Oxidation hydrogen peroxide | Generally modest change; drug regarded as comparatively robust to oxidative stress | [24] |
| Light exposure ICH Q1B | Little change reported for solid state; solution behaviour less well characterised | [25, 26] |
| Column and particle size | Sub-2 µm hybrid C18, 50 to 100 mm, giving short gradient runs and high efficiency | [12, 13] |
| Detection | Photodiode array near 250 nm, with peak purity; mass spectrometry for identification | [23, 27] |
| Gradient and temperature | Shallow ramp around parent elution; column held near 30 to 40 °C for reproducibility | [15, 28] |
| Specificity | Resolution from degradants, placebo and process impurities is the defining requirement | [9, 29] |

| Item | Reported finding or typical feature | Ref. |
|---|---|---|
| Accuracy and precision | Recovery at three levels; relative standard deviation below about 2 per cent for assay | [9, 27] |
| Robustness | Deliberate variation of flow, pH, temperature and gradient start; dwell volume affects transfer | [29, 30] |
| System suitability | Resolution mixture, tailing, plate count and replicate precision checked before each run | [16, 30] |

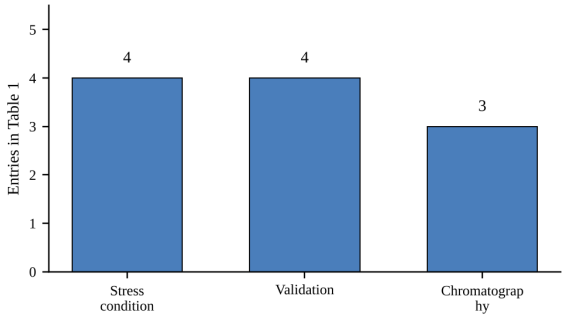



**Fig. 1: Number of entries in table 1 by topic group**

Photostability is handled separately. ICH Q1B describes confirmatory exposure of the drug substance and product to defined doses of visible and near-ultraviolet light, with dark controls to separate thermal from photochemical effects, and the photochemistry of drugs is treated in specialist monographs [26]. Because the chlorothiophene and phenyl chromophores absorb in the ultraviolet range, photolysis cannot simply be assumed away. Sample presentation affects the outcome noticeably: a thin layer of powder, a solution in a quartz cell and an intact film-coated tablet will each respond differently, and the protocol should say which was used. Many published rivaroxaban studies report solid-state light exposure only, so solution photolysis, where reactions are usually faster, is less well characterised [17, 25]. Packaging is the practical outcome of this work. If light truly affects the tablet, the blister or bottle must protect it, and the stability protocol should include the marketed pack rather than only open dishes.

## 4. UPLC METHOD DEVELOPMENT

Most reported UPLC methods for rivaroxaban use a reversed-phase column of 50 to 100 mm length and 2.1 mm internal diameter, packed with bridged ethylsiloxane/silica hybrid or similar sub-2 µm C18 material [12, 13]. The hybrid particle is popular because it tolerates a wide pH range, which is helpful when acidic degradants must be pushed into either their ionised or neutral form. Mobile phases are typically aqueous buffer or dilute acid with acetonitrile or methanol, run as a gradient, and detection is by photodiode array near 250 nm, where the drug has a strong absorbance maximum. Choosing the organic modifier changes selectivity as well as strength, so both solvents deserve a trial during screening [15, 16]. Column length has to be balanced against dwell volume and the pressure rating of the system, since a 100 mm column of 1.7 µm particles at a high flow rate can approach the instrument limit, especially with viscous methanol mixtures [12, 28].

Buffer choice is a smaller decision than it looks but still easy to get wrong. Formic acid or ammonium formate at low millimolar strength suits both UV and mass spectrometric work, while phosphate gives sharper peaks and a quieter baseline at low wavelengths but fouls ion sources and may precipitate when the organic fraction rises in a steep gradient [15, 16]. For a neutral parent drug the buffer mainly serves the degradants, holding acidic products in a defined state of ionisation so that retention times do not wander from day to day. Mobile phase should be filtered, degassed and prepared fresh, because aged aqueous buffer supports microbial growth and slow drifts in pH that show up first as shifting retention of the early peaks. Short columns also shorten equilibration between gradient runs, which adds up across a long sequence.

Gradient design is where the work is. Starting at low organic content gives retention to the polar hydrolysis fragments, and a shallow ramp across the region where rivaroxaban elutes separates it from neighbouring peaks, followed by a steeper wash for late-eluting dimers or process impurities. Column temperature near 30 to 40 °C reduces backpressure and improves mass transfer, but it also shifts selectivity and must be controlled within a narrow window [15, 28].

The practical target set out in textbook treatments is resolution of at least 2.0 between every adjacent pair, a tailing factor under 2.0 for the main peak, and a run short enough to be useful without sacrificing the ability to see impurities near the 0.05 to 0.10 per cent reporting level [7, 30]. Flow rates of 0.3 to 0.6 mL/min are usual on a 2.1 mm column, with injection volumes of one or two microlitres to limit band broadening, although small volumes put more weight on autosampler precision [13, 16].

Detection needs more thought than a single wavelength. A photodiode array detector allows peak purity to be assessed by comparing spectra across the upslope, apex and downslope of the rivaroxaban peak, and a pure peak shows matching spectra within the noise [11, 27]. The limits of this approach are often forgotten. Spectrally similar co-eluting species, which is what one expects from degradants that retain the chromophore, can pass a purity test, while low-level co-elution under a large peak can be masked by noise. For that reason, reviewers of stability-indicating methods increasingly recommend orthogonal confirmation, for example a second column chemistry, a different pH, or mass spectrometric detection [23, 25]. In practice a purity angle below the purity threshold is accepted as evidence of a single component, but software from different vendors computes these values differently, and the number is no substitute for judgement about the chromatogram [16, 27].

Mass spectrometry plays its main part in identification. Coupling the UPLC to a quadrupole time-of-flight or ion-trap instrument gives accurate masses of the degradants and, through fragmentation, structural proposals that can be matched to the hydrolytic cleavages predicted from the molecule [7, 23]. A practical constraint is that phosphate buffers, convenient for UV work, are incompatible with electrospray ionisation, so the final quality control method and the identification method often differ in their buffer. Showing that retention order is preserved between the two systems, or running a bridging experiment, avoids the situation where a peak named in the mass spectrometric work cannot be traced to a peak in the release method.

## 5. VALIDATION AND SYSTEM SUITABILITY

Validation of a stability-indicating UPLC procedure follows the framework of ICH Q2(R1), which sets out specificity, linearity, range, accuracy, precision, detection and quantitation limits, and robustness [9]. Specificity is the characteristic that earns the label stability-indicating, and it is demonstrated by showing resolution of the parent from every stress-generated peak, from placebo components, and from known process impurities [9, 29]. For linearity, published rivaroxaban methods generally describe a range spanning from the quantitation limit to about 120 or 150 per cent of the nominal assay concentration for impurity work, with correlation coefficients above 0.999 reported as normal. A correlation coefficient alone says little about linearity, and residual plots are a better check [27, 29]. Detection and quantitation limits are commonly estimated from signal-to-noise ratios of about 3 and 10, or from the slope and residual deviation of the calibration line, and the quantitation limit should sit at or below the reporting threshold for impurities [7, 9].

Accuracy for the assay is usually shown by recovery of drug added to placebo at three levels, often 50, 100 and 150 per cent of target, while accuracy for impurities uses spiked impurity standards at levels around the specification limit [9, 29]. Precision is split into repeatability, taken as six replicate preparations or nine determinations across the range, and intermediate precision, which varies day, analyst and instrument. Where relative standard deviations are reported in the rivaroxaban literature, they cluster well below the 2 per cent usually accepted for the assay. Impurity precision is naturally looser at low levels, and expecting assay-grade precision at the quantitation limit is unrealistic [9, 27]. Reproducibility between laboratories is rarely reported, and cross-laboratory transfer studies would give firmer confidence than within-laboratory repeatability alone.

Robustness is the least glamorous and arguably the most useful element for a UPLC

method. Small deliberate changes in flow rate, column temperature, buffer pH, organic composition at the start of the gradient and detection wavelength are made, and the effect on critical resolution, tailing and retention is recorded [15, 29]. Because UPLC dwell volume differs between manufacturers, a gradient that works on one instrument may shift on another, and the compendial chapter on chromatography allows defined adjustments to gradient conditions and column dimensions during transfer, within stated limits [28, 30]. Documenting how far each parameter can move before resolution is lost gives the receiving laboratory the information it actually needs. A pragmatic approach to adjustment helps here: gradient conditions can be translated to a different instrument by scaling gradient segments to column volumes, which keeps the elution profile constant even when dwell volume or column dimensions differ.

## 6. LIMITATIONS AND FUTURE DIRECTIONS

Several weaknesses recur in this literature. Many papers declare a method stability-indicating on the basis of resolution and peak purity alone, without reporting mass balance, so it is unclear whether all the lost parent drug has been accounted for as detected degradants [11, 24, 25]. Stress conditions are rarely harmonised, which makes degradation percentages impossible to compare across studies. Few papers test finished tablets from different manufacturers, so excipient interactions, which can change the pathway, are underexplored. And relative response factors for degradants are seldom determined, which means that reported impurity percentages assume equal detector response and may misstate real levels [27]. Methods developed for the drug substance are often applied unchanged to tablets, although film coating, lactose and cellulose excipients may add their own peaks or adsorb the analyte during extraction [17, 23].

The chiral dimension deserves mention. Rivaroxaban is a single enantiomer, and the (R)-enantiomer is a specified impurity in pharmacopoeial and regulatory documents, but it is invisible on an achiral reversed-phase column [7, 14]. A genuinely complete stability programme therefore needs a separate chiral method, or at least evidence that racemisation does not occur under stress. The literature on that point is thin. In practice, most laboratories deal with the issue by controlling the enantiomeric purity of the starting material and verifying it at release, an approach that is reasonable provided the oxazolidinone stereocentre is chemically unlikely to epimerise under the conditions studied. Chiral stationary phases based on polysaccharide derivatives are the usual choice for oxazolidinones, though a method fast enough to sit alongside the UPLC assay would be welcome.

Future work could usefully move in three directions. First, development guided by quality-by-design principles, with a defined design space for column, pH and gradient, would make the robustness evidence more systematic than the one-factor-at-a-time approach that dominates current reports [15, 16]. Second, wider adoption of mass spectrometric peak purity and of routine mass balance would strengthen the claim of stability-indication. Third, reporting stress conditions in a more uniform way, along the lines suggested for generic stress testing, would allow findings from different groups to accumulate [24, 25]. None of these requires exotic technology. They require agreement on what a complete study looks like. Cost and training matter too. Smaller laboratories may lack a UPLC system, and for them a validated HPLC method run at lower pressure remains a sensible, if slower, choice. Training of bench analysts is a further variable, because gradient UPLC leaves little room for sloppy sample preparation or poorly maintained seals and frits.

## 7. CONCLUSION

Published work on rivaroxaban supports a coherent picture. The molecule is vulnerable mainly to hydrolysis at its amide-type linkages, tolerates oxidation and heat better, and can be separated from its degradants on sub-2 µm reversed-phase columns with gradient elution and photodiode array detection, as summarised in table 1 and fig. 1. The technical ingredients of a good method are not in dispute. They are well described in the guidelines and in the general literature on

stress testing [6, 9, 17]. Where a laboratory must choose, the method that best resolves hydrolytic products from the parent peak, and proves it with spectra and spiked samples, deserves preference over one that is merely quick [10, 12]. Such reviews are an aid to method selection, not a substitute for local verification [6, 9].

What differs between papers is the completeness of the evidence. Mass balance, orthogonal peak purity, chiral control and finished-product studies are reported unevenly, and stress conditions vary enough to frustrate comparison [24, 25, 27]. A reader choosing a method for routine use should look for those elements specifically, and developers should include them as standard. With that discipline, UPLC offers a fast and dependable stability-indicating assay for an anticoagulant whose quality has direct clinical weight [1, 2, 29]. Until reporting practice converges, the safest course is to read each published method against ICH expectations rather than accept the label on the title page, and to repeat the key specificity experiments before relying on it for a new formulation [6, 9].

## 8. ABBREVIATIONS

UPLC-Ultra-performance liquid chromatography, HPLC-High-performance liquid chromatography, ICH-International Council for Harmonisation, API-Active pharmaceutical ingredient, UV-Ultraviolet, PDA-Photodiode array, LC–MS-Liquid chromatography–mass spectrometry, RSD-Relative standard deviation, CYP-Cytochrome P450, USP-United States Pharmacopeia

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## 10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## 11. CONFLICT OF INTERESTS

Declared none

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