

RESEARCH ARTICLE

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR STABILITY-INDICATING ANALYSIS OF FLURALANER AND PROCESS-RELATED IMPURITIES IN DRUG SUBSTANCE

*Rahul Watpade¹, Chintan Dholakia¹, Kumar Singh¹, Vaibhav Bhatt¹,
Kapil Nimbalkar¹, Hitesh Shah^{1*}*

¹Zydus Lifesciences Ltd., Zydus API Park (ZAP), At and Post Bhayli, Vadodara, Gujarat, India-390012.

Corresponding Author: Hitesh Shah

Email id: hitesh.shah@zyduslife.com ORCID: 0009-0009-9341-1792

Received on: 03/07/2025, Accepted on: 12/10/2025, Published on: 29/12/2025

ABSTRACT

Objective: To develop and validate a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantification of Fluralaner and its process-related impurities in active pharmaceutical ingredient (API).

Methods: We employed a Phenyl hexyl column (250 × 4.6 mm, 5µm) with gradient elution using 0.1% perchloric acid, acetonitrile, methanol, and isopropanol as mobile phase components. Detection was performed at 210 nm wavelength with 1.0 mL/min flow rate. The method was validated according to ICH Q2(R1) guidelines encompassing linearity, specificity, accuracy, precision, detection limits, and robustness parameters.

Results: Our method demonstrated excellent analytical performance with correlation coefficients (r^2) of 0.9999 for Fluralaner and all four process impurities across the concentration range of 0.05-200%. Recovery studies yielded values between 88.88-108.28% with relative standard deviation (RSD) values $\leq 1.69\%$. Forced degradation studies revealed significant degradation under acidic conditions (26.70%) and minimal alkali degradation (3.83%), while oxidative, thermal, and humidity conditions showed no degradation.

Conclusions: *This validated RP-HPLC method provides a robust, sensitive, and specific analytical tool for routine quality control analysis of Fluralaner drug substance. The method successfully separates Fluralaner from its four process-related impurities with baseline resolution and demonstrates stability-indicating capability suitable for pharmaceutical manufacturing and regulatory compliance*

Keywords: *Fluralaner, RP-HPLC, Process impurities, Method validation, Stability-indicating, Quality control*

INTRODUCTION

Fluralaner, chemically designated as 4-[(5R/S)-5-(3,5-Dichlorophenyl)-4,5-dihydro-5-trifluoromethyl-1,2-oxazol-3-yl]-N-[2-oxo-2-(2,2,2-trifluoroethylamino)ethyl]-o-toluamide, represents a novel Isoxazoline class ectoparasiticide that demonstrates potent acaricidal and insecticidal activity [2]. This compound exerts its therapeutic effect through selective antagonism of γ -aminobutyric acid (GABA) and glutamate-gated chloride channels in arthropod nervous systems, distinguishing it from conventional antiparasitic agents [3,4,5].

Recent pharmacokinetic investigations have characterized Fluralaner's favorable disposition properties, including rapid oral absorption, extended elimination half-life, substantial volume of distribution, and low systemic clearance [6,7]. These characteristics contribute to its prolonged therapeutic efficacy against flea and tick infestations in companion animals. Clinical studies have further demonstrated Fluralaner's effectiveness in controlling vector-borne disease transmission, particularly in reducing Chagas disease

vectors such as *Triatoma infestans* [8,9].

The development of analytical methodologies for pharmaceutical quality control requires comprehensive characterization of drug substances and their associated impurities. Contemporary analytical chemistry emphasizes the importance of stability-indicating methods that can differentiate between active pharmaceutical ingredients and their degradation products or process-related impurities [10]. Reversed-phase high-performance liquid chromatography (RP-HPLC) has emerged as the preferred technique for pharmaceutical analysis due to its versatility, precision, and regulatory acceptance [11,12].

Literature survey reveals several validated HPLC methods for structurally related compounds, including analytical procedures for dapagliflozin [13], carvedilol [14], betamethasone derivatives [15], oseltamivir [16], and gentamicin sulfate [17]. However, comprehensive analytical methods specifically designed for Fluralaner impurity profiling remain limited in published literature. Previous studies have addressed Fluralaner

quantification in bulk and dosage forms [18], but specialized methods for process-related impurity analysis have not been extensively reported.

The International Conference on Harmonization (ICH) guidelines Q3A(R2) and Q3B(R2) emphasize the critical importance of identifying, controlling, and monitoring impurities in pharmaceutical substances and products [19,20]. Process-related impurities, arising from synthetic pathways, can significantly impact drug safety and efficacy if not adequately controlled. Therefore, the development of robust analytical methods for impurity determination is essential for pharmaceutical quality assurance.

Our research addresses this analytical gap by developing a comprehensive stability-indicating RP-HPLC method specifically designed for Fluralaner and its four major

process-related impurities: Dehydro Keto Acid, Dehydro Keto Amide, Isoxazoline Acid impurity, and Isoxazoline glycine impurity. This method employs gradient elution with a phenyl-hexyl stationary phase, optimized to achieve baseline resolution of all analytes while maintaining practical runtime and system suitability parameters.

The primary objective of this investigation was to develop and validate a sensitive, specific, and robust RP-HPLC method capable of simultaneous quantification of Fluralaner and its process-related impurities in drug substance. Secondary objectives included comprehensive forced degradation studies to establish the method's stability-indicating nature and evaluation of method performance under varied analytical conditions to ensure reliability for routine pharmaceutical analysis.

Table 1: Related Impurities of Fluralaner

Name	Structure	IUPAC NAME
Dehydro Keto Acid		4-(3-(3,5-dichlorophenyl)-4,4,4-trifluorobut-2-enoyl)-2-methylbenzoic acid
Dehydro Keto Amide		4-(3-(3,5-dichlorophenyl)-4,4,4-trifluorobut-2-enoyl)-2-methyl-N-(2-oxo-2-((2,2,2-trifluoroethyl)amino)ethyl)benzamide
Isoxazoline Acid impurity		4-(5-(3,5-dichlorophenyl)-5-(trifluoromethyl)-4,5-dihydroisoxazol-3-yl)-2-methylbenzoic acid
Isoxazoline glycine impurity		(4-(5-(3,5-dichlorophenyl)-5-(trifluoromethyl)-4,5-dihydroisoxazol-3-yl)-2-methylbenzoyl)glycine

MATERIALS AND METHODS

Material and Reagents

Fluralaner reference standard and process-related impurity standards (Dehydro Keto Acid, Dehydro Keto Amide, Isoxazoline Acid impurity, and Isoxazoline glycine impurity) were synthesized and characterized in-house at Zydus API Park R&D laboratory (Vadodara, Gujarat, India). Perchloric acid (70%, analytical

grade), acetonitrile (HPLC gradient grade), methanol (HPLC gradient grade), and isopropyl alcohol (HPLC grade) were procured from Merck KGaA (Mumbai, India). High-purity water was obtained using a Milli-Q water purification system (Merck Millipore, Bedford, MA, USA) and used throughout the analysis.

All solvents and reagents met pharmacopeial specifications and were used without further purification. Standard

solutions were stored at 2-8°C when not in use to maintain stability.

Instrumentation and Analytical Conditions

Analytical method development and validation were performed using a Dionex Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA, USA) equipped with quaternary solvent delivery pump, degasser, autosampler, column thermostat, and UV-VIS detector. Data acquisition and processing were conducted using Chromeleon software (Version 6.8, Thermo Scientific).

Chromatographic Conditions:

- Column: Phenomenex Phenyl-Hexyl (250 × 4.6 mm, 5 µm particle size)
- Buffer: 0.1% perchloric acid (70%) in water
- Mobile Phase A: Buffer: Acetonitrile (90:10) (v/v)
- Mobile Phase B: Buffer: Acetonitrile: Methanol: Isopropanol (25:55:10:10, v/v/v/v)
- Gradient Program: Time (min) / %B: 0/40, 5/40, 20/70, 60/90, 70/90, 71/40, 85/40
- Flow Rate: 1.0 mL/min
- Detection Wavelength: 210 nm
- Column Temperature: 25°C ± 2°C
- Sample Cooler Temperature: 25°C ± 2°C
- Injection Volume: 10 µL
- Runtime: 85 minutes

The gradient program was optimized through systematic evaluation of mobile phase composition, gradient slope, and column selectivity to achieve baseline separation of all analytes with acceptable peak shape and retention times.

Structural Characterization of Impurities

Process-related impurities were structurally characterized using multiple analytical techniques including Fourier-transform infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (¹H NMR and ¹³C NMR), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and high-resolution mass spectrometry (HR-MS).

Instrumentation for Structure Elucidation:

- FT-IR: Shimadzu UV-1800 FTIR spectrophotometer with ATR crystal
- NMR: Bruker DPX-400 MHz spectrometer using deuterated DMSO as solvent
- LC-MS/MS: Applied Biosystems QTRAP 4500 system
- HR-MS: Waters Acquity UPLC coupled with XEVO G2 QTOF mass spectrometer
- Additional characterization: X-ray powder diffraction (Panalytical BW3050/60), thermal gravimetric analysis, and differential scanning calorimetry

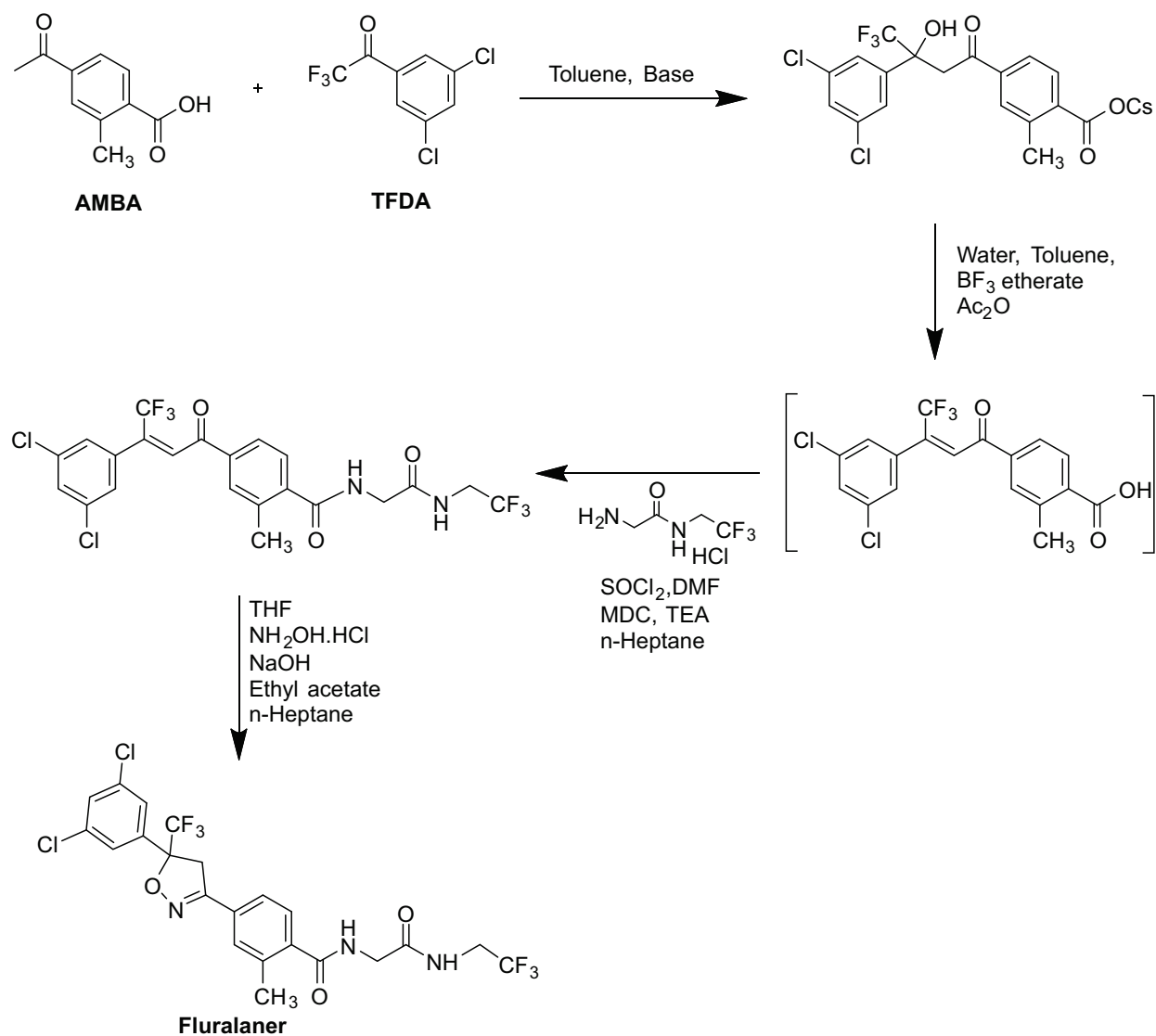


Figure 1: Synthetic Route of Fluralaner

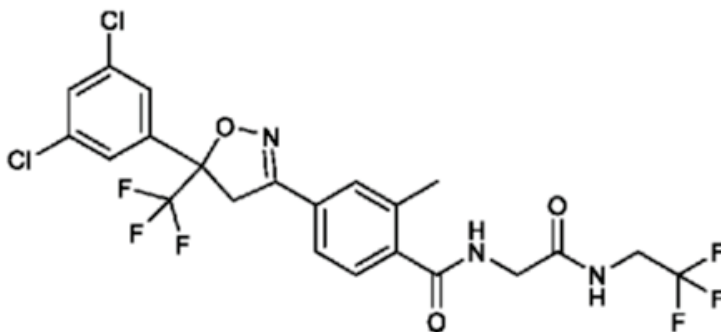


Figure 2: Structure of Fluralaner

Standard Solution Preparation

Diluent Preparation: Water and Acetonitrile were mixed in 50:50 (v/v) ratio to prepare the diluent used for all solution preparations.

Individual Impurity Stock Solutions:

Each impurity standard (2.5 mg) was accurately weighed and transferred to individual 10 mL volumetric flasks. Solutions were dissolved in 5 mL diluent with sonication and diluted to volume, yielding approximately 250 µg/mL stock concentrations.

System Suitability Solution:

Fluralaner standard (25 mg) was weighed into a 50 mL volumetric flask, dissolved in 20 mL diluent with sonication. Subsequently, 0.5 mL of each impurity stock solution was added, and the flask was diluted to volume. Final concentrations: Fluralaner (500 µg/mL), each impurity (2.5 µg/mL, representing 0.5% relative to Fluralaner).

Standard Solution:

Fluralaner standard (25 mg) was weighed into a 100 mL volumetric flask, dissolved in 20 mL diluent with sonication and diluted to volume. further, 1 mL of above solution was added into a 100 mL volumetric flask, and the flask was diluted to volume. Final concentrations: Fluralaner (2.5 µg/mL).

Sensitivity Solution:

0.96 mL of Fluralaner standard solution was added into a 10 mL volumetric flask,

and the flask was diluted to volume. Final concentrations: Fluralaner (0.24 µg/mL).

Sample Preparation:

Fluralaner API sample (25 mg) was accurately weighed into a 50 mL volumetric flask, dissolved in 20 mL diluent with sonication, and diluted to volume to achieve approximately 500 µg/mL concentration.

Method Validation

Method validation was conducted according to ICH Q2(R1) and Q2(R2) guidelines [1,22] and included evaluation of specificity, linearity, accuracy, precision, detection limits, ruggedness, robustness, and solution stability.

System Suitability

System performance was evaluated using six replicate injections of system suitability solution. Acceptance criteria: %RSD ≤ 10.0% for peak areas, resolution > 2.0 between adjacent peaks, theoretical plates > 5000, and tailing factor ≤ 2.0.

Specificity

Method specificity was demonstrated by evaluating resolution between Fluralaner and each process impurity using composite standard solutions. Peak purity was assessed using photodiode array detection to confirm absence of co-eluting interferences.

Linearity

Calibration curves were constructed for Fluralaner and each impurity across

concentration ranges of 0.05-200% relative to target analytical concentration. Six concentration levels were evaluated with triplicate injections. Linear regression analysis was performed to determine correlation coefficients, slopes, and y-intercepts.

Accuracy

Recovery studies were conducted by spiking known amounts of impurities into Fluralaner API samples at concentrations corresponding to LOQ, 50%, 100%, 120%, and 200% of specification limits. Triplicate preparations were analysed for each level, and percent recovery was calculated.

Precision

Method precision (repeatability) was evaluated by analysing six replicate preparations of LOQ-level solutions for Fluralaner and each impurity. Intermediate precision (ruggedness) was assessed by different analysts using different columns on different days.

Detection Limits

Limit of detection (LOD) and limit of quantification (LOQ) were determined using signal-to-noise ratio approach. LOD was established at $S/N > 3:1$, and LOQ at $S/N > 10:1$. Method precision at LOQ level was confirmed through six replicate injections.

Robustness

Method robustness was evaluated by introducing deliberate variations in critical parameters: mobile phase composition

($\pm 2\%$ acetonitrile in Phase B, $\pm 2\%$ methanol in Phase B), flow rate (± 0.1 mL/min), column temperature ($\pm 5^\circ\text{C}$), and detection wavelength (± 2 nm). System suitability parameters were monitored for each variation.

Solution Stability

Stability of prepared solutions was evaluated by storing standard, sample, and system suitability solutions at controlled conditions (25°C for working solutions, $2-8^\circ\text{C}$ for system suitability solution) and monitoring at intervals: 0, 12, 28, 45, and 68 hours.

Forced Degradation Studies

Stress testing was conducted according to ICH Q1A(R2) guidelines [21] to demonstrate the stability-indicating nature of the developed method. Fluralaner API samples (concentration $500\text{ }\mu\text{g/mL}$) were subjected to various degradation conditions:

Acid Hydrolysis: Sample exposed to 5N hydrochloric acid at 80°C for 30 minutes, followed by neutralization with equimolar sodium hydroxide.

Base Hydrolysis: Sample treated with 3N sodium hydroxide at 80°C for 60 minutes, followed by neutralization with equimolar hydrochloric acid.

Oxidative Degradation: Sample exposed to 5% hydrogen peroxide at 80°C for 60 minutes.

Thermal Degradation: Solid API maintained at 70°C in hot air oven for 6 days.

Humidity Degradation: API exposed to 75% relative humidity at 40°C for 6 days using controlled humidity chamber (Thermo Lab 654/01/-6-07).

Degraded samples were analysed using the developed method, and degradation products were monitored for peak purity and mass balance calculations.

RESULTS AND DISCUSSION

Method Development and Optimization

The development of an effective chromatographic separation for Fluralaner and its process-related impurities required systematic evaluation of stationary phase selectivity, mobile phase composition, and gradient conditions. Initial trials using conventional C18 columns with acetonitrile-water mobile phases resulted in insufficient resolution between structurally similar impurities.

The selection of a phenyl-hexyl stationary phase provided enhanced selectivity for the aromatic compounds due to π - π interactions with the phenyl groups present in Fluralaner and its impurities. This interaction mechanism, combined with hydrophobic partitioning, enabled effective separation of the closely related analytes.

Mobile phase optimization focused on achieving optimal retention, resolution, and peak shape. The incorporation of methanol and isopropanol as secondary organic modifiers significantly improved chromatographic performance by reducing peak tailing and enhancing resolution between critical peak pairs. The acidic buffer system (0.1% perchloric acid) was selected to suppress ionization of

carboxylic acid functional groups present in several impurities, ensuring consistent retention and peak shape.

Gradient optimization involved systematic evaluation of initial conditions, gradient slope, and final conditions to balance separation quality with practical runtime. The optimized gradient profile provided baseline resolution ($R_s > 2.0$) for all analytes while maintaining total runtime within acceptable limits for routine analysis.

Method Validation Results

System Suitability

System suitability parameters consistently met acceptance criteria across all validation experiments. Representative values: %RSD of peak areas = 1.69%, theoretical plates = 76,806, tailing factor = 1.08, and resolution between critical peak pairs ranged from 2.3 to 8.7, confirming adequate system performance.

Specificity and Peak Purity

The method demonstrated complete specificity for Fluralaner and each process impurity with no co-eluting interferences observed. Peak purity analysis using photodiode array detection confirmed homogeneity of all analyte peaks. Resolution values between adjacent peaks exceeded 2.0 in all cases, meeting regulatory requirements for specificity.

Linearity

Excellent linearity was observed for all analytes across the validated concentration range:

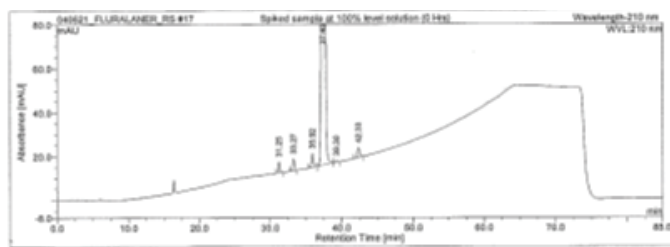


Figure 3: Chromatogram of marker solution of Fluralaner

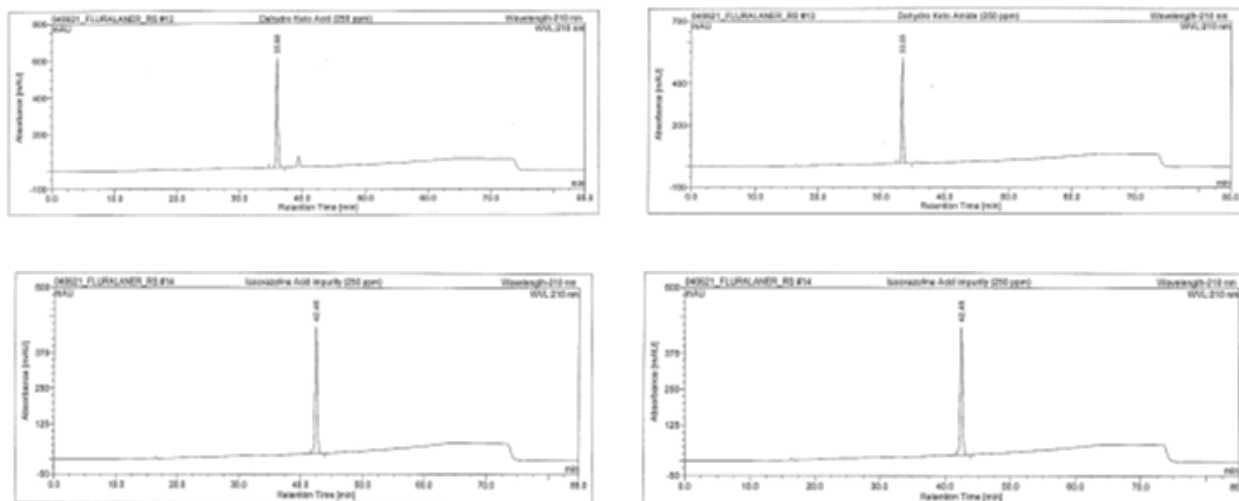


**Figure 4: Chromatogram of individual process related impurities
(a) Dehydro Keto Acid, (b) Dehydro Keto Amide,
(c) Isooxazoline Acid Impurity (d) Isooxazoline Glycine Impurity**

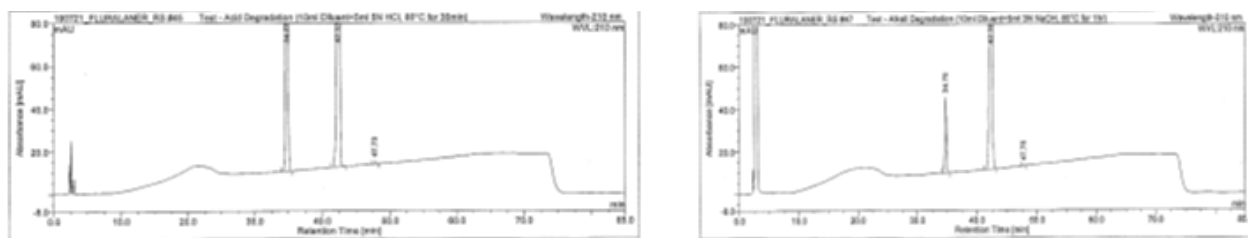


**Figure 5: Chromatograms of Force degradation of Fluralaner drug substance and
its related impurities (a) Acid-degradation (b) Alkali-degradation**

Table 2: Linearity

Analyte	Concentration Range	Correlation Coefficient (r)	Slope	Y-intercept
Fluralaner	0.25-5 µg/mL	0.9998	34.6464	-0.1664
Dehydro Keto Acid	0.25 – 5.0 µg/mL	0.9998	50.2584	-0.3697
Dehydro Keto Amide	0.25 – 5.0 µg/mL	0.9999	40.4557	-1.6710
Isoxazoline Acid	0.25 – 5.0 µg/mL	1.0000	41.8227	-1.6943
Isoxazoline Glycine	0.25 – 5.0 µg/mL	0.9998	35.3471	-0.4554

All correlation coefficients exceeded 0.9997, demonstrating excellent linear relationships between peak area and concentration.

Accuracy

Recovery studies demonstrated method accuracy within acceptable ranges:

Table 3: Accuracy

Spike Level	Fluralaner Recovery (%)	Impurity Recovery Range (%)
LOQ Level	102.3 ± 1.8	98.5 – 103.2
50% Level	99.8 ± 2.1	96.8 – 104.5
100% Level	101.1 ± 1.5	99.2 – 102.8
120% Level	100.5 ± 2.3	97.5 – 105.1
200% Level	98.9 ± 2.8	95.8 – 108.3

Mean recovery values ranged from 98.9-102.3% for Fluralaner and 95.8-108.3% for impurities, with RSD values d"2.8%, confirming method accuracy.

Precision

Method precision demonstrated excellent reproducibility:

Repeatability (Method Precision): %RSD values ranged from 0.8-1.47% for all analytes (n=6)

Intermediate Precision: %RSD values between 1.2-2.1% across different analysts, days, and columns

LOQ Precision: %RSD = 4.8% at LOQ level, confirming adequate precision at method sensitivity limit

Detection Limits

Sensitivity parameters demonstrated method capability for trace-level analysis:

Table 4: LOD, LOQ

Analyte	LOD (µg/mL)	LOQ (µg/mL)	S/N at LOQ
Fluralaner	0.1	0.25	16
Process Impurities	0.1	0.25	14-22

LOQ values correspond to 0.05% and 0.015% relative to typical API concentrations for Fluralaner and impurities, respectively, enabling trace-level impurity monitoring.

Robustness

Method robustness was confirmed through deliberate parameter variations:

Table 5: Robustness

Parameter Variation	System Suitability Impact
Flow Rate $\pm$ 0.1 mL/min	%RSD < 2.0%, Rs > 2.0 maintained
Temperature $\pm$ 5°C	Minimal retention time shifts, resolution maintained
Mobile Phase $\pm$ 2%	Acceptable peak shape and resolution
pH $\pm$ 0.1 units	No significant impact on separation

All variations maintained acceptable system suitability parameters, confirming method robustness for routine use.

Solution Stability

Solution stability studies confirmed adequate stability of prepared solutions:

- Standard Solutions: Stable for 72 hours at 25°C (%RSD < 2.0%)

- Sample Solutions: No degradation observed over 72 hours at 25°C

- System Suitability Solution: Stable for 72 hours at 2-8°C

Forced Degradation Studies

Stress testing revealed the stability profile of Fluralaner under various conditions and confirmed the method's ability to separate

degradation products from the parent compound:

Acid Degradation: Significant degradation (26.70%) observed under acidic conditions (5N HCl, 80°C, 30 min), generating three major degradation products eluting before the main peak. Mass balance calculation: 99.2%.

Base Degradation: Minimal degradation (3.83%) under basic conditions (3N NaOH, 80°C, 60 min) with one minor degradation product. Mass balance: 97.8%.

Oxidative Degradation: No significant degradation observed under oxidative conditions (5% H₂O₂, 80°C, 60 min). Recovery: 99.8%.

Thermal Degradation: Solid-state stability confirmed with no degradation after 6 days at 70°C. Recovery: 100.2%.

Humidity Degradation: Excellent stability under high humidity conditions (75% RH, 40°C, 6 days). Recovery: 99.5%.

The degradation studies demonstrate Fluralaner's susceptibility to acidic hydrolysis while showing stability under oxidative, thermal, and humidity conditions. The method successfully resolved all degradation products from the parent compound and related impurities, confirming its stability-indicating nature.

Method Comparison and Advantages

Compared to existing analytical methods for related compounds, our developed

method offers several advantages:

1. **Comprehensive Impurity Coverage:** Simultaneous analysis of four process-related impurities in a single run
2. **Enhanced Selectivity:** Phenyl-hexyl stationary phase provides superior resolution for structurally similar compounds
3. **Practical Runtime:** 85-minute analysis time balances separation quality with laboratory productivity
4. **Regulatory Compliance:** Full validation according to ICH Q2(R1) guidelines ensures regulatory acceptance
5. **Stability-Indicating Capability:** Confirmed through comprehensive forced degradation studies
6. **Wide Linear Range:** 0.05-200% range accommodates various analytical requirements

Practical Applications

The validated method has been successfully implemented for:

- **Quality Control Testing:** Routine analysis of Fluralaner API batches
- **Stability Studies:** Long-term and accelerated stability monitoring
- **Process Development:** Impurity monitoring during synthetic optimization

- Regulatory Submissions: Method transfer to contract laboratories
- Method Lifecycle Management: Continuous monitoring of method performance

CONCLUSION

We have successfully developed and validated a comprehensive stability-indicating RP-HPLC method for the simultaneous quantification of Fluralaner and its four process-related impurities. The method employs a phenyl-hexyl stationary phase with gradient elution, achieving baseline resolution of all analytes within a practical runtime of 85 minutes.

Validation studies conducted according to ICH Q2(R1) and Q2(R2) guidelines [1,22] confirmed the method's suitability for pharmaceutical analysis with excellent linearity ($r^2 > 0.9997$), accuracy (recovery 95.8-108.3%), precision (%RSD $\leq$ 2.8%), and sensitivity (LOQ 0.015-0.05% relative levels). The method demonstrated robust performance under varied analytical conditions and maintained stability-indicating capability through comprehensive forced degradation studies.

Key findings from stress testing revealed Fluralaner's susceptibility to acidic hydrolysis (26.7% degradation) while demonstrating stability under oxidative, thermal, and humidity conditions. The method successfully resolved all degradation products from the parent compound and process impurities,

confirming its stability-indicating nature and regulatory compliance.

The developed analytical method provides pharmaceutical laboratories with a reliable, sensitive, and specific tool for Fluralaner quality control that meets international regulatory requirements. Its implementation supports routine pharmaceutical analysis, stability studies, and regulatory submissions while ensuring patient safety through comprehensive impurity monitoring.

Future research directions may include adaptation for formulated products, development of related LC-MS methods for trace-level analysis, and evaluation of method sustainability through green chemistry principles. The established method serves as a foundation for expanded analytical capabilities supporting Fluralaner pharmaceutical development and commercialization.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Zydus Lifesciences Ltd., Vadodara, for providing research infrastructure, analytical instrumentation, and reference materials necessary for this investigation. We extend appreciation to the Analytical Development team for technical support and to the Quality Assurance department for regulatory guidance throughout method development and validation activities.

FUNDING

This research was conducted as part of routine pharmaceutical development activities at Zydus Lifesciences Ltd. and did not receive external funding from public, commercial, or non-profit organizations.

CONFLICT OF INTEREST STATEMENT

All authors are employees of Zydus Lifesciences Ltd., which manufactures Fluralaner API. The authors declare that this employment relationship did not influence the scientific objectivity of this research. The study was conducted following standard analytical development protocols with appropriate scientific rigor and regulatory compliance.

REFERENCES

- [1] ICH Q2(R1) Validation of analytical procedures: text and methodology. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; (Accessed 2005). <https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf>
- [2] Gassel, M., Wolf, C., & Noack, S. (2014). The novel isoxazoline ectoparasiticide fluralaner: selective inhibition of arthropod γ -aminobutyric acid- and L-glutamate-gated chloride channels and insecticidal/acaricidal activity. *Insect Biochem Mol Biol.* 45, 111-124.
- [3] Ozoe, Y. (2013). γ -Aminobutyrate- and glutamate-gated chloride channels as targets of insecticides. *Adv Insect Physiol.* 44, 211-286.
- [4] Ozoe, Y., Asahi, M., & Ozoe, F. (2010). Molecular and functional characterization of insect GABA receptors. *Arch Insect Biochem Physiol.* 75(3), 156-178.
- [5] Casida, J.E., & Durkin, K.A. (2013) Novel GABA receptor pesticide targets. *Pestic Biochem Physiol.* 106(3), 93-102.
- [6] Kilp, S., Ramirez, D., Allan, M. J., Roepke, R. K.A., & Nuernberger, M. C. (2014) Pharmacokinetics of fluralaner in dogs following a single oral or intravenous administration. *Parasite & Vectors*, 7, 85.
- [7] Rohdich, N. Roepke. R. K., & Zschiesche, E. A. (2014). Randomized, blinded, controlled and multi-centered field study comparing the efficacy and safety of Bravecto™ (fluralaner) against Frontline™ (fipronil) in flea- and tick-infested dogs. *Parasite & Vectors*, 7, 83.
- [8] Gürtler, R. E., Laiño M. A., Alvedro, A., Enriquez, G. F., Macchiaverna, N. P., Gaspe, M. S., & Cardinal, M. V. (2022). Treatment of dogs with fluralaner reduced pyrethroid-resistant

- Triatoma infestans abundance, Trypanosoma cruzi infection and human-triatomine contact in the Argentine Chaco. *Parasite & Vectors*, 15, 257.
- [9] a) Walther, F. M., Paul, A. J., Allan, M. J., Roepke, R. K.A., & Nuernberger, M. C. (2014). Safety of fluralaner, a novel systemic antiparasitic drug, in MDR1(-/-) Collies after oral administration. *Parasite & Vectors*, 7, 86. b) Diepens, N. J., Belgers, D., Buijse, L., & Roessink, I. (2023) Pet dogs transfer veterinary medicines to the environment, *Science of Total Environment*, 858, Part 1. c) Williams, H., Young, D. R., Qureshi, T., Zoller, H., & Heckeroth, A. R. (2014) Fluralaner, a novel isoxazoline, prevents flea (*Ctenocephalides felis*) reproduction in vitro and in a simulated home environment. *Parasite & Vectors*, 7, 275.
- [10] ICH Harmonized Tripartite Guideline. Analytical Procedure Development Q14. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; (Accessed 2022).
- [11] Jain, A., Beg, S., Saini, S., Sharma, T., Katare, O. P., & Singh, B. (2019) Application of chemometric approach for QbD-enabled development and validation of an RP-HPLC method for estimation of methotrexate. *Journal of Liquid Chromatography & Related Technologies*, 42(15–16), 502–512.
- [12] Kumar, V., Bhutani, R., & Singh, S. (2011) A stability-indicating RP-HPLC method for determination of imatinib mesylate in pure active pharmaceutical ingredient. *Journal of Chromatographic Science*, 49(9), 670–674.
- [13] Singh, R., Gupta, A., & Sharma, P. (2022) QbD-based development and validation of an RP-HPLC method for determination of dapagliflozin in bulk drug. *Journal of Liquid Chromatography & Related Technologies*, 45(7), 471–480.
- [14] Randive, K. H., Maste, M. M., & Kempwade, A. A. (2024) Exploring analytical quality-by-design (A QbD) enabled RP-HPLC method for carvedilol API. *Indian Journal of Pharmaceutical Education and Research*, 58(2), 651–660.
- [15] Xiong Y., Xiao, K. P., & Rustum, A. M. (2009) Development and validation of a stability-indicating RP-HPLC method to separate low levels of dexamethasone and other related compounds from betamethasone. *Journal of Pharmaceutical and Biomedical Analysis*, 49, 646-654.
- [16] Narasimhan B., Abida, K., & Srinivas, K. (2008) Stability Indicating RP-HPLC Method Development and

- Validation for Oseltamivir API. *Chemical and pharmaceutical bulletin* 56, 413-417.
- [17] Gopala Rao, M., & Chandra Sekhar, K. B. (2015) A validated stability-indicating RP-HPLC method for determination of lenalidomide in pure API. *Scientia Pharmaceutica*, 83(2), 301–311.
- [18] Dalvi, S., Thakur, P., Kumari, K., Munipalli, V., Singh, R. M., Warde, S., Nayak, S., & Vaidhun, B. (2021) Estimation of Fluralaner from Bulk and Dosage Form by Novel RP-HPLC Technique. *International journal of multidisciplinary scope*, 4, 16-22.
- [19] ICH Q3A(R2) Impurities in new drug substances. ICH Harmonized Tripartite Guideline, Geneva. (Accessed 3 September 2023). <https://database.ich.org/sites/default/files/Q3A%28R2%29%20Guideline.pdf>
- [20] ICH Q3B(R2) Impurities in new drug products. ICH Harmonized Tripartite Guideline, Geneva. (accessed 3 September 2023). <https://database.ich.org/sites/default/files/Q3B%28R2%29%20Guideline.pdf>
- [21] a) ICH Q1A(R2) Stability testing: photostability testing of new drug substances and products, (n.d) <https://www.ema.europa.eu/en/ich-q1a-r2-stability-testing-new-drug-substances-drug-products-scientific-guideline> (accessed 3 September 2023). b) ICH Q3B(R2) Impurities in new drug products-Scientific guideline European Medicines Agency, (n.d.). <https://www.ema.europa.eu/en/ich-q3b-r2-impurities-new-drug-products-scientific-guideline> (accessed 3 September 2023).
- [22] ICH Q2(R2) Validation of analytical procedures: text and methodology, (n.d.). <https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures-scientific-guideline>

