Research Article

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ACLIVIA: a novel rapid testing kit for pesticide residue in green tea leaf

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Abstract

BACKGROUND: Ensuring the accurate trace-level detection of pesticide residues in tea leaves is crucial for maintaining safety, meeting international trade standards and promoting sustainable agricultural practices. A study was undertaken to validate the performance of ACLIVIA, an AI-powered molecular recognition platform developed by Arogyam Medisoft Solution Pvt Ltd, in detecting residues of monocrotophos, acephate, acetamiprid, imidacloprid, dinotefuran and fipronil in freshly plucked tea leaves. These pesticides account for *ca* 90% of the total non-compliance of made tea in India.

RESULTS: ACLIVIA requires 10 min for sample preparation and 2–4 min for analysis for identification of these pesticides. The validation was conducted through laboratory studies at TLabs, Tea Research Association, Kolkata, as well as field trials at tea estates managed by Luxmi Tea Company. A total of 332 samples, with pesticide residue levels ranging from 0 to 0.1 mg kg^{-1}, were tested. ACLIVIA demonstrated high efficiency, achieving 94.12–100% sensitivity, 94.4–100% specificity, 95.52–96.92% accuracy and with a limit of detection at 0.005 mg kg^{-1}. Additionally, the platform showed minimal interference from other pesticides. Field trials further confirmed that semi-skilled workers could be trained to operate the analyzer effectively within couple of days, making it suitable for use at factory level.

CONCLUSION: These results highlight ACLIVIA's potential to revolutionize the tea industry by offering a fast, affordable (Rs150 or US$1.70 per sample testing cost) and reliable solution for detecting pesticide residues in green tea leaves.

Keywords: ACLIVIA; pesticide residue; MRL; rapid testing kit; green tea leaf

INTRODUCTION

Ensuring food safety has become a priority in modern agriculture due to the growing awareness of the adverse health effects associated with pesticide residues in food products. Pesticides, while essential for protecting crops from pests and diseases, often leave behind residues that can exceed permissible levels, posing risks to consumers and hindering trade compliance with stringent regulatory standards.[1] Conventional methods for detecting pesticide residues, such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS), are highly sensitive and reliable but require sophisticated infrastructure, skilled personnel and considerable time for analysis.[2,3] These limitations make them less practical for on-site testing and rapid decision-making, particularly in resource-constrained settings.

In response to these challenges, rapid detection kits have emerged as a practical solution, offering simplicity, portability and speed. These kits enable the rapid screening of food products for pesticide residues without the need for extensive laboratory setups, making them particularly useful for field-level applications. They leverage diverse technologies, including immunoassays, colorimetric methods and biosensors, to provide qualitative or semi-quantitative results in minutes.[4] For instance, lateral flow immunoassays use antibody-based reactions to detect specific pesticides, while enzyme inhibition assays target classes like organophosphates by measuring their impact on enzymatic activity.[5] A critical review of the available screening methods for pesticide residues on the basis of optical detection during the period 2016–2020 was conducted by Tsagkaris *et al.* in 2021.[6] Optical biosensors paved the way for introducing the point-of-care (POC) era.[7,8] Colorimetry, fluorescence, surface plasmon resonance and surface-enhanced Raman spectroscopy techniques have been reviewed. Use of nanomaterials which can significantly enhance optical detection performance and handheld platforms,[9-11] the hyphenation of optical assays to smartphones was also underlined due to user friendliness and ease of getting results features such as one-click results using smartphone apps or online result communication. All in all, despite being in an early stage and facing several challenges, that is, long sample preparation protocols or interphone variation results, such POC diagnostics pave a new road into the food safety field in

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which analysis cost will be reduced and more intensive testing will be achieved.

Despite their advantages, rapid detection kits face challenges such as limited sensitivity, cross-reactivity and the inability to detect a broad spectrum of pesticides simultaneously.[12] Nevertheless, advancements in nanotechnology, biosensor design and smartphone-integrated platforms are improving the accuracy and usability of these kits, enabling them to meet the increasing demand for real-time pesticide residue analysis.[13,14] A detailed comparison of different aspects between rapid testing kits like ACLIVIA and traditional residue analysis equipment like LC–MS/MS and GC–MS/MS is summarized in Table 1.

Tea, a globally consumed beverage, faces stringent quality standards to meet safety and trade regulations.[15-17] Pesticide residue testing in the tea industry predominantly focuses on made tea, which is the traded commodity subject to regulatory scrutiny. The maximum residue limit (MRL) is established by regulatory authorities in different countries to ensure that pesticide residues in tea are within safe and acceptable levels for consumers.[18] However, the process of manufacturing made tea from green tea leaves involves a range of practices tailored to the type of tea being produced, such as black, green, oolong or white tea. These manufacturing practices, which often include withering, rolling, fermenting and drying, can significantly influence the concentration of pesticide residues in the final product.

The variation in pesticide residue levels between green tea leaves and made tea can be attributed to two key factors. First, residue degradation occurs due to the application of high temperatures during processing, leading to the breakdown of certain pesticide compounds. Second, biomagnification may occur because the manufacturing process reduces moisture content in tea leaves from approximately 78% to about 3–4%, concentrating the residues in the made tea.[19] This variability underscores the importance of accurate and early detection of pesticide residues at the green tea leaf stage.

Detecting pesticide residues in green tea leaves before they enter the manufacturing process offers several advantages. Early detection allows for informed decision-making, such as segregating non-conforming batches to prevent them from being processed, thereby saving production costs. This approach is particularly relevant in the context of bought leaf factories, where small tea growers collectively sell their green leaf harvest. A single batch with non-conforming pesticide levels can compromise the compliance of the entire lot, leading to financial and reputational losses for the producers.[20] By identifying non-conforming batches at the green leaf stage, the remaining leaf can be safeguarded and processed in compliance with regulatory requirements.

In this context, the detection of pesticide residues at trace levels in green tea leaves is an urgent necessity for the Indian tea industry. Regulatory agencies, such as the Food Safety and Standards Authority of India (FSSAI), have set stringent MRLs for certain commonly detected pesticides, including acetamiprid, imidacloprid, acephate, monocrotophos, fipronil and dinotefuran, at levels as low as 10 ng g^{-1}.[16] Achieving this level of sensitivity in detection is technically challenging, especially in field settings.

Innovative detection methods that are sensitive, rapid and capable of analyzing pesticide residues at the green tea leaf stage are crucial for ensuring regulatory compliance and maintaining

Table 1. Advantages and disadvantages of rapid testing methods compared to conventional methods

Aspect	Rapid testing kit (ACLIVIA)	GC–MS	LC–MS
Speed	Provides results within 15 min	Time-consuming; takes hours for complete analysis	Time-consuming but slightly faster than GC–MS
Portability	Lightweight, battery-operated and field-deployable	Requires a laboratory setting and bulky equipment	Laboratory-based, not portable
Ease of use	User-friendly; minimal training required	Requires skilled personnel for operation and interpretation	Requires highly trained personnel
Sensitivity and accuracy	High sensitivity and accuracy as compared to GC–MS and LC–MS	High accuracy for volatile and semi-volatile compounds	Highly sensitive and ideal for a wide range of pesticides
Throughput	Suitable for high-throughput screening	Lower throughput due to longer sample preparation and analysis time	Moderate throughput with complex sample preparation
Sample preparation	Minimal preparation required	Extensive sample preparation and extraction steps	Requires complex sample preparation
Cost	Lower initial investment and operational costs	High equipment and operational costs	Very expensive equipment and maintenance
Detection capability	Can detect single and multiple pesticides with a high specificity and in a very short time	Best for volatile and semi-volatile pesticide residues	Suitable for polar, non-volatile and thermally unstable pesticides
Regulatory compliance	Suitable for on-site preliminary screening	Compliant with regulatory standards for legal enforcement	Compliant with global food safety regulations
Maintenance and consumables	Requires very little maintenance; reagents have a limited shelf life	Expensive maintenance; requires high-purity gases, columns and solvents	High maintenance costs; needs solvents, columns and high-purity gases
Best use case	Ideal for rapid field testing and preliminary screening	Best for confirmatory analysis of volatile pesticides. Not ideal for onsite testing	Best for confirmatory analysis of non-volatile pesticides. Not ideal for onsite testing

the competitiveness of Indian tea in global markets. Addressing these challenges will not only enhance food safety but also support the livelihoods of small tea growers, who form a significant part of the industry. By investing in early detection technologies, the tea industry can mitigate risks, improve sustainability and ensure consumer trust.

ACLIVIA (Fig. 1) is a lightweight (approximately 500 g), easy-to-use, single-platform analyzer equipped with artificial intelligence (AI; computer vision and machine learning)-based molecular recognition capabilities for rapid detection (2–30 min) of pesticide residues in green tea leaf. The platform has a smartphone-based user interface. ACLIVIA, developed by Arogyam Medisoft Solution Pvt Ltd, based on technologies jointly developed with Centre for Development of Advanced Computing, Kolkata, supported by Ministry of Electronics and Information Technology, was validated at TLabs, Tea Research Association (TRA), Kolkata and field-verified at Fulbari tea estate of Luxmi Tea Company.

ACLIVIA for green tea leaf currently has the ability to detect residues of monocrotophos, fipronil, acetamiprid, imidacloprid, acephate and dinotefuran up to 0.01 mg kg^{-1}. In this paper, we discuss the salient features of ACLIVIA, a rapid detection kit designed for pesticide residue analysis in green tea leaves. This technology has been validated for detecting pesticide residues at trace levels, aligning with stringent regulatory standards such as the 0.01 mg kg^{-1} MRL set by FSSAI. Additionally, we present the validation results that demonstrate its effectiveness and reliability for early-stage detection. Finally, we explore the challenges that remain in the field, including the need for further advancements in sensitivity, specificity and scalability for broader applications in the tea industry.

Unique features of ACLIVIA

Integration of multiple recognition systems

The system (Fig. 2) integrates various recognition materials based on host–guest chemistry, nanoparticles, metal–organic framework and quantum dot enhancing accuracy and versatility. For detecting monocrotophos, fipronil, acetamiprid, imidacloprid, acephate and dinotefuran in fresh tea leaves, each standard operating procedure (SOP) utilizes two distinct sets of reagents, which are clearly labeled reagent A and reagent B. Reagent A is used to initiate alkaline hydrolysis within a pH range of 12–14, while reagent B serves as recognition material, formulated using gold nanoparticles. For field use, these reagents are dispensed using small dropper bottles equipped with a calibrated drop mechanism. The bottles resemble standard eye drop bottles in appearance. The reagents can be stored at normal temperature and in dark, dry and cool spaces.

Figure 1. ACLIVIA device.

Combinations of colorimetric and fluorescent capture systems

Colorimetric and fluorometric capture mechanisms are combined into a unified system enhancing versatility. Colorimetric capture system in ACLIVIA for Tea includes an LED-based illumination system with emission wavelengths ranging from 400 to 750 nm and CMOS (complementary metal-oxide semiconductor) image sensors.[21] The variation in the colorimetric signal, produced during the reaction between the recognition material and the pesticide residue under ongoing alkaline hydrolysis,[22] has been observed to differ distinctly based on the pesticide class and residue concentration.[23]

AI-driven analysis system

The system employs computer vision and machine learning to interpret image-based signals and chemical interactions. The ACLIVIA analyzer uses a proprietary algorithm developed using a multilayer perceptron model[24] based on artificial neural networks to process reaction kinetics data derived from the interaction between the recognition material and the pesticide extract. This algorithm converts the processed data into a qualitative result, using a computed threshold that factors in both the pesticide concentration and its class to determine the presence and level of pesticide residues. The proprietary algorithm uses one hidden layer with different neurons and sigmoid function boundary stimulus functions and different training methods. The post-propagation error method with the Levenberg–Marquardt algorithm[25] is used for faster convergence in network training.

Cloud-enabled smartphone interface

The system has the ability to transmit information to a cloud-based server, whenever it is connected to the internet. This testing platform can be used in remote areas.

MATERIALS AND METHODS

Materials

Detecting monocrotophos, fipronil, acetamiprid, imidacloprid, acephate and dinotefuran in fresh tea leaves requires the use of two different extraction methods using deionized distilled water as the solvent and three distinct SOPs. SOP 1 is designated for monocrotophos, SOP 2 for the simultaneous detection of acetamiprid, imidacloprid, acephate and dinotefuran, and SOP 3 for fipronil.

The ACLIVIA testing kit along with two sets of reagents (reagent A and reagent B) were developed by Arogyam Medisoft Solution. Analytical standards for monocrotophos, fipronil, acetamiprid, imidacloprid, acephate, dimethoate, hexythiazox, novaluron, flubendamide, hexaconazole, glyphosate, paraquat, azoxystrobin, emamectin benzoate, ethion, carbamate, 2,4-D, saflufemacil, dimethoate, hexythiazox, emamectin benzoid, abamectin, cypermethrin, deltamethrin, bifenthrin, fenvalerate and fluvalinate were procured from Dr Ehrenstorfer, LGC, UK. GC–MS/MS

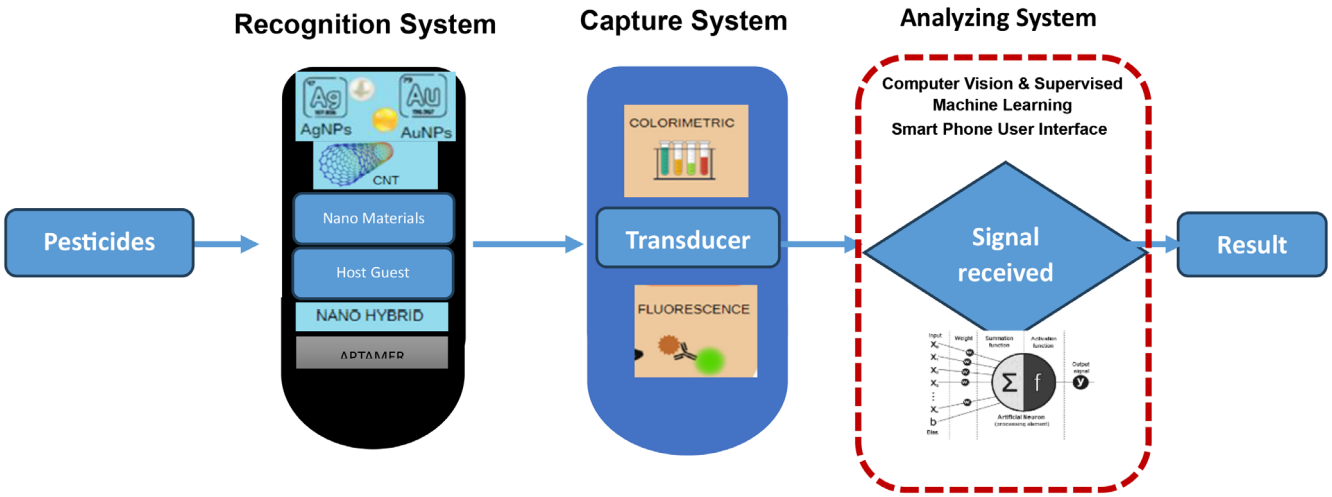

Figure 2. Overview of ACLIVIA architecture.

(7000D, Agilent) and LC–MS/MS (6460, Agilent) were utilized for quantitative checking of the spiked and field samples for confirmatory study. Fresh tea leaves were procured from different sections of Borbhetta Tea estate of TRA Tocklai plantations at Jorhat, Assam, representing different varieties. They were collected between June 2024 and September 2024 and sent to TLabs, Kolkata within 2 days from the date of plucking for the purpose of validation testing. These samples were not sprayed with any pesticide for a minimum period of last 6 months.

Sample size determination

It is estimated to perform the validation tests on a minimum of 91 samples with an estimated assumption of 95% sensitivity, 95% specificity, margin of error of 20% and prevalence of 10%.[26]

Design of the study

The fresh tea leaf samples received from TRA Tocklai plantations at Jorhat, Assam were considered negative. Received samples were spiked with different concentrations (0.001, 0.005, 0.01, 0.05 and 0.1 mg kg^{-1}) of pesticides using standards of monocrotophos, fipronil, acetamiprid, imidacloprid, acephate and dinotefuran and were kept overnight. Apart from these, control green tea leaf samples were spiked with dimethoate, hexythiazox, emamectin benzoid, dimethoate, hexythiazox, novaluron, flubendamide, hexaconazole, glyphosate, paraquat, azoxystrobin, abamectin, ethion, carbamate, 2,4-D, saflufemacil and other pesticides as per PPC (Plant Protection code) version 16 of Tea Board India listed chemicals each of 0.001, 0.005, 0.01, 0.05 and 0.1 mg kg^{-1} concentration and were kept overnight. These spiked samples were considered positive when the concentration was above 0.01 mg kg^{-1}.

Sample preparation

Deionized distilled water was used as an extraction solvent. Green tea leaf samples were weighed (1 g for SOP 1 and SOP 2; 5 g for SOP 3), cut into small pieces and placed in a 50 mL centrifuge tube. An amount of 10 mL of distilled water was poured into the tube and shaken by hand. After that, the tube was kept for 10 min at room temperature without any agitation. After that, the extract was taken out using a dropper as mentioned in the description of the SOPs. No further cleaning of the extract was done. One drop of reagent A was mixed with 3 drops of reagent B followed by 11 drops of green tea leaf extract and the reaction tube was inserted in the ACLIVIA. For monocrotophos as per SOP 1, the reading will come immediately in the system. For the detection of acephate, acetamiprid, imidacloprid and dinotefuran as per SOP 2, the reading will come after 2 min on the ACLIVIA screen. For extraction of fipronil (SOP 3), the sample weight was 5 g and the centrifuge tube was vortexed for 1 min. The reading will come after 2 min on the ACLIVIA screen (Table 2). The flow diagram of all the SOPs is summarized in Fig. 3, providing a clear overview of the experimental workflow.

Table 2. Details of three SOPs

		Extraction procedure		
SOP	For detection of	Weight of sample (g)	Use of vortex	Reaction time (min)
SOP 1	Monocrotophos	1	No	0
SOP 2	Acephate, acetamiprid, imidacloprid and dinotefuran	1	No	2
SOP 3	Fipronil	5	Yes	2

Testing using ACLIVIA

Following the instructions mentioned in the ACLIVIA instruction manual, each sample was analyzed using ACLIVIA for Tea three times a day by multiple operators over five different days. Special attention was directed at avoiding mixing of the samples. The tests were conducted at room temperature (24–26 °C) and the outcomes were documented as the actual results. Positive and negative samples were also separately analyzed using GC–MS/MS and LC–MS/MS at TLabs for further confirmation of the pesticide residues.

Statistical analysis

The actual results were compared with expected results to determine limit of detection (LOD), specificity, sensitivity and accuracy of ACLIVIA for Tea. Receiver operating characteristic (ROC) curve and area under curve (AUC) of ACLIVIA for Tea were calculated using Statistics Kingdom software, available online.[27] The Handbook on Rapid Analytical Food Testing (RAFT) Vol 1.0 (Guidelines for the

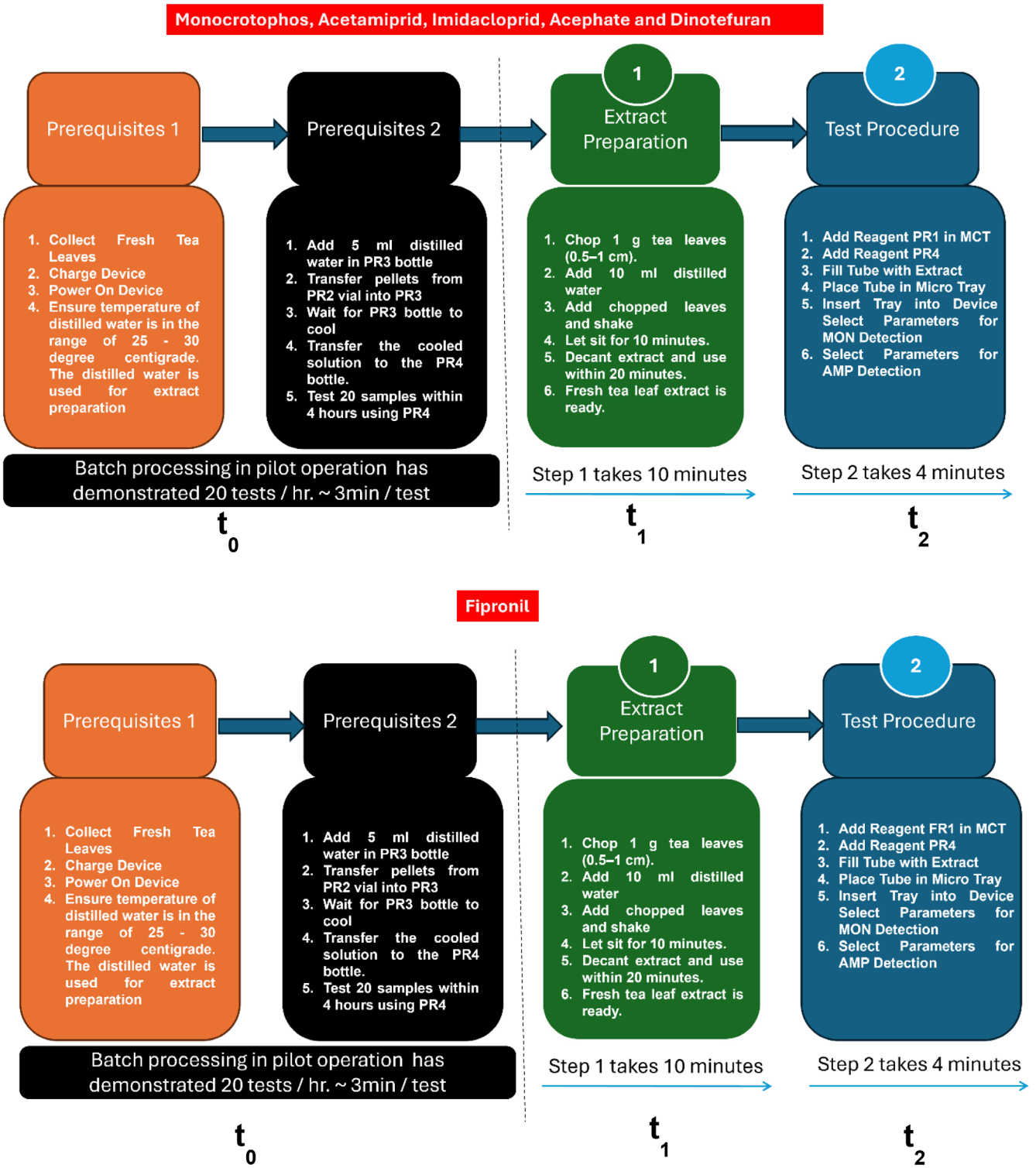

Figure 3. Rapid analysis of pesticide residues in fresh tea leaf.

Verification of RAFT KIT/Equipment/Method) was referred to for establishing the analytical benchmark and statistical analysis.[28]

RESULTS

Analytical performance

The sensitivity, specificity and accuracy of the ACLIVIA kit for the three SOPs were tested during the validation study. Sensitivity, or true positive rate, quantifies how well a test identifies true positives. Stated alternatively, sensitivity measures the proportion of subjects with an actual positive outcome (i.e. true positives + false negatives) who are correctly given a positive assignment (i.e. true positives only). Specificity, or true negative rate, quantifies how well a test identifies true negatives (i.e. how well a test can classify subjects who truly do not have the condition of interest). Stated alternatively, specificity measures the proportion of subjects with

Table 3. Key performance outcomes of different SOPs

SOP	Number of tests (*N*)	True positive (TP)	True negative (TN)	False positive (FP)	False negative (FN)	Sensitivity (%)	Specificity (%)	Accuracy (%)	LOD (mg kg^{-1})
SOP 1	195	32	157	4	2	94.12	97.52	96.92	0.005
SOP 2	67	42	22	0	3	93.18	100.00	95.52	0.005
SOP 3	30	12	17	1	0	100.00	94.44	96.0	0.005

Sensitivity = TP/(TP + FN); specificity = TN/(TN + FP); accuracy = (TP + TN)/*N*. SOP 1: rapid identification of monocrotophos. SOP 2: rapid identification of acephate, acetamiprid, imidacloprid and dinotefuran. SOP 3: rapid identification of fipronil.

an actual negative outcome (i.e. true negatives + false positives) who are correctly given a negative assignment (i.e. true negatives only). Accuracy is also used as a statistical measure of how well a binary classification test correctly identifies or excludes a condition. That is, the accuracy is the proportion of correct predictions (both true positives and true negatives) among the total number of cases examined. The details of validation results of ACLIVIA at TLabs, TRA are presented in Table 3.

A total of 195 tests were done for SOP 1, 67 tests for SOP 2 and 30 tests for SOP 3. The LOD, the lowest analyte concentration that was likely to be reliably distinguished from the blank response, for all three SOPs was 0.005 mg kg^{-1}. The sensitivity of SOP 3 was found to be the highest (100%) followed by SOP 1 (94.12%) and then SOP 2 (93.18%). The highest specificity (100%) was achieved in SOP 2 followed by SOP 1 (97.52%) and SOP 3 (94.44%). In the case of accuracy, SOP 1 showed 96.92%, SOP 2 presented 95.52% and SOP 3 indicated 96%. So, all the SOPs demonstrated notable efficiency and accuracy in detecting pesticide residues in green tea leaves.

The highly efficient performance of the three SOPs can be explained from the algorithm model set for each of the SOPs based on the training set data. From the ROC curves presented in Fig. 4, it transpired that the AUC values for all three SOPs (SOP 1: 0.9183; SOP 2: 0.8503; SOP 3: 0.915) were close to 1 and the curve passed through the upper left corner which means that the predictability of the model is quite high.

Findings from field trials

After validating the analytical performance of ACLIVIA for green tea leaves at TLabs, TRA, Kolkata, a field trial was conducted at two tea estates of Luxmi Tea Company in Assam and West Bengal to assess its usability, performance metrics, reliability and applicability in tea production. During the trial, 14 field operators were successfully trained, highlighting the device's user-friendliness and minimal learning curve. Most operators were able to perform the test independently after a brief hands-on session, typically within a few hours.

Different sets of plants from the two plantations were used at this stage. Those included plants sprayed with pesticides permissible as plant protection code and plants specifically sprayed with commercial formulations of monocrotophos, fipronil, acephate, acetamiprid, imidacloprid and dinotefuran. Leaves from these plants were used for testing to check if specific ACLIVIA tests gave positive signal with the leaves specifically sprayed with the named pesticides and negative signal for all other leaves. In addition to these leaves, a few leaves obtained from other sources were used randomly to check if any of these leaves provided a positive signal. The date of spraying, date of plucking, date of testing and the ambient temperature were recorded. At this stage, the stability of the sample extracts was evaluated to determine how long they could consistently reproduce the same results.

These tests were performed on 90 tea leaf samples in three different time periods between August 2024 and September 2024.

The following key observations were noted during the field trial:

(I) All the tests correctly differentiated the sprayed samples from non-sprayed samples when tested within the first 3 days of plucking. This verified the tests have high sensitivity during field use.
(II) No fixed-temperature incubator was used during the tests, and it was observed that the reaction time varied depending on the ambient temperature. Based on this finding, a change was introduced to the SOP, emphasizing the need for recalibrating ACLIVIA for Tea to account for changes in agro-climatic conditions.
(III) Sample extracts were found to remain stable and produce consistent results for up to 30 min. However, no verification of their stability beyond this time frame was performed.
(IV) The sample extracts produced 100% reproducible results, when tested by the primary investigator from Arogyam Medisoft. Subsequent testing revealed variations when tested by a newly trained field operator at Luxmi Tea. When tested by a newly trained field operator at Luxmi Tea a second time, the reproducibility dropped to 94% for SOP 1 and to 88% for SOP 2.
(V) One plantation reported buyer rejections dropped to zero after implementing ACLIVIA.
(VI) The plantations reported a testing throughput of 20 trucks per hour at bought-leaf factory gates.

Feedback from the field indicated that real-time use of ACLIVIA required minor operational adaptations, particularly in sample preparation under varying environmental conditions such as temperature and ambient light. These observations led to refinements in the standard operating protocol, ensuring greater consistency and accuracy in diverse field settings. Overall, the field trial validated ACLIVIA's robustness, ease of deployment and user adaptability, while also highlighting opportunities for future optimization and contextual calibration based on environmental factors.

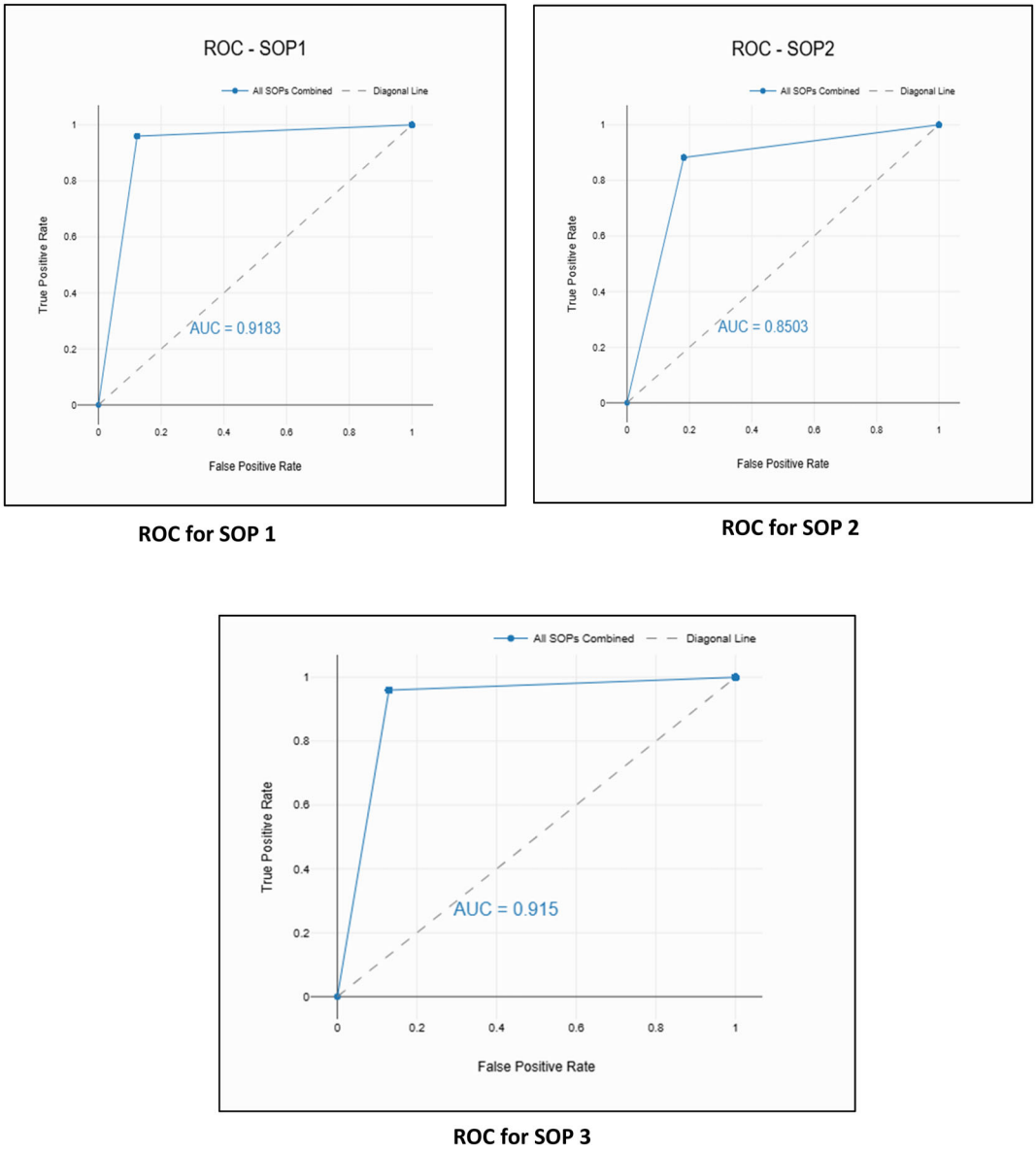

Figure 4. ROC curves for the three SOPs.

DISCUSSION

ACLIVIA represents a groundbreaking advancement in pesticide residue detection by overcoming the challenges associated with traditional methods such as GC–MS or LC–MS. These conventional techniques, while highly precise, are resource-intensive, requiring expensive equipment, skilled personnel and significant processing time, thereby limiting their accessibility and field applicability. In contrast, ACLIVIA offers a portable, rapid and cost-effective solution with accuracy and specificity that rival those of laboratory-grade instruments. This makes it particularly advantageous for the tea industry, where real-time detection is crucial for ensuring compliance with international safety standards, maintaining product quality and reducing the financial burden of outsourcing testing to external laboratories. Furthermore, the efficiency of its AI-driven design reduces the dependency on extensive operator training and streamlines integration into existing workflows.

Thus far, six pesticides – acetamiprid, imidacloprid, monocrotophos, acephate, dinotefuran and fipronil – have been validated using ACLIVIA in green tea leaves at a detection level of 0.005 mg kg^{-1}. However, according to PPC Version 16 of the Tea Board of India, 42 pesticides are allowed for use in Indian tea plantations, and these six pesticides are not registered for tea by the Central Insecticides Board & Registration Committee. Despite this, internal surveillance reports from the Tea Board, FSSAI, research organizations like TRA as well as export and auction houses indicate that these six pesticides account for approximately 90% of non-compliance cases under the default MRL set by FSSAI. This makes ACLIVIA a critical tool for addressing key areas of concern, providing better clarity for these non-compliance issues. While the current scope of validation is significant, there is an ongoing need to expand its capabilities to cover additional pesticides, a goal actively being pursued.

In this context, in India, the analysis of pesticide residues has predominantly relied on advanced techniques such as GC–MS/MS and LC–MS/MS. These methods are highly sensitive and capable of detecting a wide range of pesticide compounds. We have also tested some non-spiked and spiked samples at different levels (0.01 to 0.10 mg kg^{-1}) for all three SOPs and subsequently tested using ACLIVIA as well as LC–MS/MS and GC–MS/MS. The results are depicted in Table 4. The results in Table 4 clearly demonstrate that ACLIVIA provides comparable detection capability to traditional LC–MS/MS and GC–MS/MS methods for monocrotophos, acephate, acetamiprid, imidacloprid, dinotefuran and fipronil at various spiking levels. Notably, ACLIVIA consistently identified positive samples at low spiking levels (10–100 μg kg^{-1}), matching the detection ranges of conventional instruments. Control samples were correctly reported as negative, confirming the method's specificity. The slight variations in measured concentrations between ACLIVIA and standard equipment remain within acceptable analytical limits, supporting ACLIVIA's potential as a reliable field-level screening tool for pesticide residues in tea leaves. To address the need for more accessible and rapid testing methods, alternative approaches have been explored.

A biosensor kit (Biokit) for detection of organophosphate and organocarbamate pesticides has been developed by Bhabha Atomic Research Center.[29] The kit can detect the presence of 12 insecticides in the soil samples, water resources and in food commodities (vegetables, fruits and spices). The Biokit has been recognized as a rapid food testing kit by FSSAI in a press release (31 December 2019). However, this kit is not validated or recommended for tea to test for banned pesticides like fipronil, acetamiprid, imidacloprid, dinotefuran, acephate and cypermethrin. Moreover, the LOD is very high for most of the parameters that can be tested using the Biokit.

One such development is the Pesticide Detection Kit by the Defence Food Research Laboratory in Mysore. This kit is designed for on-site testing and can detect various pesticide residues in food and environmental samples without the need for complex laboratory equipment.[30] But this kit has been developed for use with vegetables and fruits, not validated on tea. Beyond this, no major advancements in India have emerged until ACLIVIA. Most other efforts continue to rely on high-end analytical methods like GC–MS and LC–MS. In this regard, Tata Consumer Products Ltd supported the development of a pesticide detection kit for tea by the National Institute of Food Technology Entrepreneurship and Management in Kundli. The kit can detect major pesticide residues in tea within 30–60 min.[31]

ACLIVIA has been validated for the residue range of 0–0.10 mg kg^{-1} of the selected pesticides. The residue level at field-applied recommended dose will be much higher and beyond the calibration range of this device. Samples need to be diluted and brought within the calibration range to get accurate results. So, primarily, ACLIVIA is recommended for trace-level analysis. However, expansion of the dynamic range should be the scope of future research. The adaptability and broad detection range of ACLIVIA present a significant advancement in the field of on-site pesticide residue monitoring. Designed for rapid and simultaneous detection of multiple pesticide classes – including organophosphates, neonicotinoids and fungicides – ACLIVIA can also be used across diverse agricultural matrices such as green tea leaves, cumin, coriander, fennel and fenugreek. Its robust performance in field-level environments, combined with minimal sample preparation and a rapid detection time of 4–10 min, makes it especially suited for decentralized testing. This flexibility not only reduces reliance on centralized laboratory infrastructure but also enables timely decision-making for both producers and regulators. The platform's scalability further allows for the inclusion of additional analytes or matrices, making ACLIVIA a promising tool for integrated pesticide residue surveillance and compliance with food safety standards.

ACLIVIA has also been successfully deployed and validated across more than 18 tea gardens, where it is being actively used at the factory gate to screen incoming green leaves for pesticide contamination. Based on the results provided by ACLIVIA, the gardens are able to segregate contaminated leaves at the entry point, ensuring that only clean, residue-free leaves are used for manufacturing made tea. This proactive approach has significantly improved quality control, with several customers reporting a complete elimination of made tea rejections due to pesticide residues. Furthermore, many users have taken the initiative to

Table 4. Comparison of results between ACLIVIA and traditional residue analysis equipment (LC–MS/MS and GC–MS/MS)

SOP	Pesticides detected	Spiked level (μg kg^{-1})	No. of samples	ACLIVIA results		LC–MS/MS results (mg kg^{-1})	GC–MS/MS results (mg kg^{-1})
				Positive	Negative		
SOP 1	Monocrotophos	Control	10	—	10	ND	—
		10	10	9	1	0.008–0.009	—
		50	10	9	1	0.044–0.047	—
		100	10	10	—	0.091–0.096	—
SOP 2	Acephate, acetamiprid, imidacloprid and dinotefuran	Control	10	—	10	ND	—
		10	10	9	1	0.007–0.009	—
		50	10	10	—	0.045–0.048	—
		100	10	10	—	0.086–0.095	—
SOP 3	Fipronil	Control	10	—	10	—	ND
		10	10	10	—	—	0.008–0.010
		50	10	10	—	—	0.045–0.052
		100	10	10	—	—	0.091–0.11

SOP 1 and SOP 2 pesticides analyzed in LC–MS/MS and SOP 3 pesticide analyzed in GC–MS/MS.
ND, not detected.

independently validate ACLIVIA's performance through NABL-accredited laboratories, consistently finding its results to be highly reliable and accurate. These field-level outcomes affirm ACLIVIA's effectiveness, usability and impact in improving pesticide compliance in tea production.

Despite its advantages, the ACLIVIA pesticide residue analyzer has certain limitations that merit consideration. Although ACLIVIA has been successfully validated for detecting pesticide residues in fresh tea leaves, its modular and adaptable design opens the door to broader applications, including monitoring soil and water quality and extending its use to other agricultural products. However, challenges persist, such as sensitivity to extreme environmental conditions like temperature and humidity, and the need for further optimization to detect other pesticide compounds. Although it offers rapid and multi-residue detection, its sensitivity may not match the lower detection limits achievable by advanced laboratory-based methods such as GC–MS/MS or LC–MS/MS, particularly for trace-level contaminants. Environmental factors like extreme temperature fluctuations, dust or prolonged exposure to moisture can also affect device calibration and performance. Furthermore, while the device is designed for on-field use, consistent results depend on adequate user training and strict adherence to testing protocols. Moreover, it is currently validated only for green tea leaves and selected spices, and may require further method optimization and matrix validation before it can be reliably applied to other food commodities.

To maximize its potential, future research should focus on extending validation efforts to cover diverse tea plant clones across varying agro-climatic regions and seasonal settings, as well as processed tea types like black, green and oolong teas. Enhancing its sensitivity to detect ultra-trace levels of rare pesticides, exploring its capabilities in environmental diagnostics and conducting economic analyses (the testing cost calculated around Rs150 or US$1.70 per sample currently) to quantify its cost-effectiveness will further establish ACLIVIA as an indispensable tool for sustainable and technology-driven agriculture.

CONCLUSION

The validation of ACLIVIA at TRA highlights its transformative potential for pesticide residue detection in tea manufacturing. By integrating advanced AI technologies with molecular recognition systems, ACLIVIA provides a rapid, reliable and affordable solution. The study demonstrated its high accuracy (LOD of 0.005 mg kg^{-1}), efficiency (0–4 min per analysis) and specificity in detecting a broad range of pesticides.

ACLIVIA addresses critical challenges faced by the tea industry, enabling producers to ensure product safety, meet international standards and reduce operational costs. Its field-ready design further supports real-time decision-making and enhances quality assurance throughout the supply chain.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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