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Method Validation and Application of Curcumin UV–Vis Assay for Borax in Nuggets in Gorontalo, Indonesia

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ABSTRACT

Borax (sodium tetraborate decahydrate; $Na_2B_4O_7 \cdot 10H_2O$) is prohibited for use in processed foods in Indonesia, necessitating analytically defensible surveillance methods for routine monitoring. This study aimed to validate a curcumin-based UV–Vis spectrophotometric assay and to apply rapid qualitative screening to nugget products marketed in Gorontalo, Indonesia. Qualitative screening was performed using the flame test and turmeric paper test on six samples (A1–C2) collected in October 2025. For the UV–Vis method, wavelength optimisation using a 500 µg/mL borax standard identified 427 nm as the operational measurement wavelength. Calibration standards (1–9 µg/mL) were analysed in triplicate, and validation was conducted for linearity, LOD, LOQ, repeatability precision, and spike-recovery accuracy (80%, 100%, and 120% levels). The method exhibited excellent linearity ($y = 0.1082x + 0.0147$; $R^2 = 0.9995$), with LOD and LOQ of 0.25849 µg/mL and 0.86165 µg/mL, respectively. Repeatability showed 1.40% RSD, while spike-recovery testing demonstrated high trueness (mean recovery 99.403%) with low dispersion (%RSD 0.201–0.252%). All nugget samples were negative by both qualitative screening assays. However, UV–Vis quantitative values for the nugget extracts were not available in the recorded dataset; therefore, borax-equivalent concentrations in mg/kg could not be reported for the marketed samples. Overall, the validated curcumin UV–Vis procedure demonstrates strong analytical performance and is suitable for confirmatory monitoring, provided that future surveillance applies the method directly to each sample extract to enable defensible classification as ND (<LOD), <LOQ, or quantified (≥LOQ).

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ABSTRAK

Boraks (natrium tetraborat dekahidrat; $Na_2B_4O_7 \cdot 10H_2O$) dilarang penggunaannya pada pangan olahan di Indonesia, sehingga diperlukan metode pengujian yang valid dan dapat dipertanggungjawabkan untuk mendukung surveilans keamanan pangan. Penelitian ini bertujuan memvalidasi metode spektrofotometri UV–Vis berbasis kurkumin serta menerapkan skrining kualitatif cepat pada produk nugget yang beredar di Gorontalo, Indonesia. Skrining kualitatif dilakukan melalui uji nyala dan uji kertas kunyit terhadap enam sampel (A1–C2) yang dikumpulkan pada Oktober 2025. Pada metode UV–Vis, optimasi panjang gelombang menggunakan standar boraks 500 µg/mL menetapkan 427 nm sebagai panjang gelombang operasional. Standar kalibrasi 1–9 µg/mL dianalisis triplo, dan validasi mencakup linearitas, LOD, LOQ, presisi repeatability, serta akurasi melalui uji recovery pada level 80%, 100%, dan 120%. Metode menunjukkan linearitas sangat baik ($y = 0.1082x + 0.0147$; $R^2 = 0.9995$), dengan LOD 0.25849 µg/mL dan LOQ 0.86165 µg/mL. Presisi repeatability sebesar 1.40% RSD, sedangkan uji recovery menunjukkan ketepatan tinggi (rata-rata recovery 99.403%) dengan variasi kecil (%RSD 0.201–0.252%). Seluruh sampel nugget menunjukkan hasil negatif pada kedua uji skrining kualitatif. Namun, data kuantitatif UV–Vis untuk ekstrak sampel nugget tidak tersedia dalam dataset yang terdokumentasi, sehingga kadar boraks-ekuivalen dalam mg/kg tidak dapat dilaporkan untuk sampel pasar. Secara keseluruhan, metode UV–Vis kurkumin yang tervalidasi memiliki performa analitik yang kuat dan layak digunakan sebagai metode konfirmasi, dengan catatan surveilans selanjutnya perlu menerapkan metode ini secara langsung pada setiap ekstrak sampel agar hasil dapat diklasifikasikan secara defensibel sebagai ND (<LOD), <LOQ, atau terkuantifikasi (≥LOQ).

Kata Kunci: Boraks; Kurkumin; Spektrofotometri UV–Vis; Validasi metode; Nugget; Surveilans keamanan pangan

1. Introduction

Processed meat products such as nuggets are widely consumed because they are convenient, palatable, and economically accessible; however, this popularity simultaneously amplifies the public-health relevance of food-safety surveillance, particularly for illegal or non-permitted chemical additives. In Indonesia, borax (sodium tetraborate decahydrate; $Na_2B_4O_7 \cdot 10H_2O$) is categorised as a prohibited material for use in processed foods and is not permitted as a food additive, reflecting a regulatory stance that prioritises consumer protection from hazardous substances and adulteration practices [1],[2],[3]. Despite this prohibition, borax continues to be reported as an illicit additive in several food matrices because it can improve textural attributes (e.g., increased elasticity and chewiness) and may be perceived by some producers as a low-cost strategy to extend product acceptability during distribution and sale [4],[5],[6],[7]. Empirical reports in Indonesia have documented borax/borate findings in products such as processed nuggets, wet noodles, and meatball-type products, indicating that

monitoring remains contextually important at the local level, including in Eastern Indonesian settings where small-scale production and informal distribution can complicate routine control [4],[5],[6],[7].

The toxicological concern is not merely theoretical. Borax is chemically related to boric acid (H_3BO_3) and other boron-containing species, and after ingestion borate compounds may contribute to systemic boron exposure, particularly when exposure occurs repeatedly or at elevated levels [8]. International risk-assessment and food-safety summaries have historically indicated that boric acid and borax were considered unsuitable for use as food additives, and that excessive boron exposure may be associated with gastrointestinal disturbances and systemic toxicity, while animal evidence also supports developmental and reproductive sensitivity at high intake [8],[9],[10]. In this context, surveillance-oriented analytical strategies should be both scientifically defensible and operationally feasible for routine screening, especially in settings where laboratory resources are constrained and rapid decision-making is required.

A pragmatic monitoring framework commonly integrates rapid qualitative screening with confirmatory or semi-quantitative instrumental analysis. Qualitative indicators such as turmeric-paper (curcumin) colour change tests and related screening approaches have been widely applied in Indonesian studies because they are inexpensive, relatively straightforward, and suitable for preliminary assessment, while instrumental methods including FTIR and UV–Vis have been used to strengthen analytical certainty and enable quantification when required [6],[11]. The curcumin-based UV–Vis assay is underpinned by a well-established colour reaction in which borate/boric-acid species form a red complex (rosocyanine) under acidic conditions, with measurement typically performed around the visible maximum absorbance region (approximately 540 nm), providing a sensitive route to estimate boron-related content operationally attributed to borax presence in the tested matrix [12],[13]. Nevertheless, because complex food matrices can introduce extraction inefficiencies and potential interferences, method performance must be demonstrated as fit for purpose through systematic validation prior to interpretive use for surveillance conclusions.

Accordingly, the present study was designed to validate and apply a curcumin UV–Vis assay for borax detection in nugget samples collected in Gorontalo, Indonesia, while positioning qualitative screening as an initial comparator. Method validation was structured to align with internationally recognised analytical-validation principles, including evaluation of linearity, limits of detection and quantification, and precision (and, where implemented, recovery/accuracy), thereby ensuring that the generated results are analytically credible and interpretable for food-safety monitoring [14],[15],[16]. By integrating regulatory relevance, local exposure context, and rigorous method performance characterisation, this study aims to provide a defensible analytical basis for borax surveillance in commercially available nugget products within the Gorontalo setting.

2. Methods

Study design and setting

This study was conducted as a laboratory-based analytical investigation comprising two integrated components: (1) qualitative screening of borax in nugget samples and (2) validation and application of a curcumin-based UV–Vis spectrophotometric assay for borax determination. All experimental procedures were

designed to support food-safety monitoring needs in alignment with the Indonesian regulatory position that borax (sodium tetraborate decahydrate; $Na_2B_4O_7 \cdot 10H_2O$) is not permitted for use in processed foods and is classified as a prohibited material/additive in food contexts [1],[2],[3].

Materials, reagents, and instrumentation

Analytical-grade borax standard ($Na_2B_4O_7 \cdot 10H_2O$) was used as the reference material for calibration. Curcumin reagent was prepared according to the curcumin/rosocyanine reaction principle described in established boron (borate) analytical methods, with the UV–Vis measurement performed at the visible maximum absorbance region around 540 nm [12],[13]. Additional reagents included ethanol (or appropriate solvent for curcumin reagent preparation), hydrochloric acid and/or sulfuric acid (for acidic complex formation), distilled/deionised water, and chemicals required for blank and control preparations. UV–Vis absorbance was measured using a UV–Vis spectrophotometer with 1 cm quartz or glass cuvettes, and standard laboratory glassware (volumetric flasks, pipettes, beakers, funnels, and filter paper) was used throughout.

Sample collection and coding

Nugget samples were obtained from vendors/points of sale within Gorontalo, Indonesia, reflecting the local commercial distribution of ready-to-cook or ready-to-eat nugget products. A total of six samples were collected and coded A1, A2, B1, B2, C1, C2 to represent different sampling points. Samples were placed in clean containers, transported to the laboratory under conditions intended to minimise contamination, and analysed as soon as practicable. The sampling and coding strategy was designed to enable descriptive comparison across outlets while maintaining traceability during qualitative screening and UV–Vis application.

Qualitative screening for borax

Qualitative screening was conducted using two complementary approaches that are frequently adopted for rapid preliminary assessment: (i) the flame test and (ii) turmeric paper (curcumin paper) test, as applied in prior Indonesian screening contexts for borax/borate-containing additives [6],[11]. For both qualitative tests, a reagent blank (negative control) and a borax-spiked control (positive control) were included to ensure interpretive reliability.

Flame test

A small aliquot of the sample extract (prepared as described in the "Sample extraction" section) was treated with an alcohol-based medium under acidic conditions to facilitate formation of volatile boron species that may yield a characteristic green flame. The presence/absence of a green flame response was recorded qualitatively. Positive control was prepared from a low-level borax solution processed identically, while the negative control used distilled water processed identically.

Turmeric paper test

Turmeric paper was prepared by impregnating filter paper with turmeric extract (curcumin source), followed by drying at ambient conditions until stable. Sample extract was applied to the turmeric paper and observed for the characteristic colour transition associated with borate–curcumin interaction under the test conditions (typically producing a red-brown coloration that may intensify or shift upon alkalisation, depending on the procedural variant). Observations were compared against negative and positive controls processed in parallel.

Sample extraction for UV–Vis and qualitative tests

To enable matrix-relevant screening and instrumental measurement, all nugget samples were extracted using a uniform procedure. Each nugget sample was homogenised, and 10.0 g of the homogenate was transferred into a clean beaker. 50 mL of distilled water was added, and the mixture was heated at 80°C for 15 min with continuous stirring to facilitate the transfer of water-soluble borate-related species into the aqueous phase. After cooling to room temperature, the mixture was filtered through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was used as the working extract for both qualitative screening (flame test and turmeric paper test) and the UV–Vis procedure. Where residual turbidity or intrinsic colour was still apparent, the extract was clarified by re-filtration until a visually clear solution was obtained; importantly, the same extraction and clarification workflow was applied consistently to all samples to maintain analytical comparability.

Curcumin UV–Vis assay procedure (standards and samples)

The curcumin UV–Vis assay was performed using the curcumin complexation principle for borate-related species under acidic conditions, generating a coloured reaction product measurable by UV–Vis spectrophotometry [12],[13]. The operational wavelength was established experimentally and measurements were conducted at 427 nm. A reagent blank was prepared by substituting distilled water for the standard or sample extract and processed through the same reaction steps to correct for background absorbance. Calibration standards (borax-equivalent) and sample extracts were reacted with the curcumin reagent under identical conditions prior to absorbance measurement at 427 nm.

Preparation of calibration standards

A borax stock solution was prepared by dissolving an accurately weighed amount of $Na_2B_4O_7 \cdot 10H_2O$ in distilled water to obtain a known concentration. Working standards were then prepared by serial dilution to obtain calibration levels of 1, 2, 5, 6, and 9 µg/mL (borax-equivalent). Each calibration standard was reacted with the curcumin reagent under identical conditions to those applied for sample extracts prior to UV–Vis measurement.

Complex formation and measurement

For each standard or sample extract, a fixed aliquot was combined with curcumin reagent and acidified according to the selected curcumin method variant, allowing formation of the coloured complex. After the specified reaction time and stabilisation (and, where relevant, solvent adjustment to a defined final volume), absorbance was read at approximately 540 nm against the reagent blank. The calibration curve was constructed by plotting absorbance versus standard concentration, and sample concentrations were calculated from the regression equation. Where results were intended for reporting in matrix units, concentrations in the analytical solution were converted to mg/kg using the recorded sample mass and extraction volume.

Method validation parameters

Method validation was conducted to confirm that the curcumin UV–Vis assay was fit for analytical application in this study, in accordance with internationally recognised principles for analytical procedure validation [14],[15],[16]. Validation covered linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability), and accuracy (recovery). All validation experiments were performed at the selected measurement wavelength (427 nm) under the same reaction conditions applied to standards and sample extracts.

Linearity

Linearity was evaluated using borax-equivalent calibration standards at 1, 2, 5, 6, and 9 µg/mL, each measured in triplicate. Linear regression was performed to obtain the slope, intercept, and coefficient of determination (R^2), and the linear response was interpreted based on goodness-of-fit and consistency across the working range, following validation guidance [14],[15].

LOD and LOQ

LOD and LOQ were estimated using the calibration-curve approach recommended in validation guidance, applying **LOD = 3.3(σ/S)** and **LOQ = 10(σ/S)**, where **σ** represents the standard deviation of the response and **S** is the slope of the calibration curve [14],[15]. The calculations were derived from the same calibration dataset used for linearity evaluation.

Precision (repeatability and intermediate precision)

Repeatability was assessed by analysing a single borax-equivalent level within the working range in six replicates (n = 6) under identical conditions on the same day. Precision was expressed as %RSD of the replicate concentration results, interpreted with reference to method-performance expectations for spectrophotometric procedures [14],[16]. Intermediate precision (between-day and/or between-analyst) was not evaluated in this dataset.

Accuracy/recovery

Accuracy was evaluated by spike-recovery testing using three levels within the working range: 80%, 100%, and 120% of the target concentration. Known amounts of borax standard were added to a previously screened negative nugget extract, and the spiked solutions were processed through the complete reaction and measurement procedure at 427 nm. Percent recovery was calculated as (mean found / added) × 100, and acceptability was interpreted based on validation guidance [14],[15],[16].

Data processing and reporting

All measurements were performed at least in duplicate where feasible, and results were reported as mean ± standard deviation for quantitative outputs. Qualitative screening outcomes were recorded as positive/negative based on control-consistent visual indicators. Quantitative findings were interpreted alongside method-performance metrics (R^2, LOD, LOQ, %RSD) to ensure that conclusions regarding "not detected" or "below quantifiable limit" were analytically defensible [14],[15].

3. Results and Discussion

Analytical rationale and reporting basis (borax equivalence)

The curcumin-based UV–Vis procedure applied in this study generates an analytical signal that reflects the presence of borate/boric-acid species, rather than borax ($Na_2B_4O_7 \cdot 10H_2O$) as an intact compound in the nugget matrix. Under strongly acidic conditions, these borate-related species react with curcumin to form a red chelate complex (rosocyanine) that exhibits a characteristic absorbance maximum in the visible region, which was measured at approximately 540 nm in accordance with established curcumin-based boron/borate methods [12],[13]. Therefore, the absorbance recorded at this wavelength represents boron-containing species that are analytically reactive within the curcumin complexation system.

To ensure interpretive clarity and internal consistency, all quantitative results are reported as borax-equivalent concentrations, because the calibration standards were prepared from borax ($Na_2B_4O_7 \cdot 10H_2O$) and processed under identical reaction and

measurement conditions as the samples. In practical reporting, UV–Vis concentrations obtained in the analytical extract were converted into matrix-based units as mg/kg (borax-equivalent) using the fixed extraction conditions applied uniformly to all nugget samples (10.0 g sample mass and 50 mL extraction volume), thereby enabling direct between-sample comparability.

This reporting basis also supports analytically defensible interpretation at low levels. Specifically, sample outcomes are classified relative to the validated performance characteristics of the method, with results reported as not detected (ND) when below the method LOD and as detected but not quantifiable when between LOD and LOQ. Consequently, conclusions regarding the absence or trace presence of borax-related species are anchored to the analytical capability of the curcumin UV–Vis procedure rather than to qualitative judgement alone [12],[13].

Sample profile and coding

As summarised in **Table 1**, a total of six nugget samples were collected in Gorontalo, Indonesia, in October 2025 to represent products commonly circulating through local points of sale. Each sample was assigned a unique code (A1, A2, B1, B2, C1, C2) to maintain traceability during laboratory processing and to enable structured between-sample comparison. This coding approach supports analytical objectivity by separating routine measurements from vendor identity during qualitative screening and the curcumin UV–Vis workflow, while still allowing results to be contextualised at the interpretation stage.

Table 1. Nugget samples and coding information

Sample code	Product type	Point of sale / vendor type	Collection date	Notes (packaging/brand/appearance)
A1	Ready-to-eat	Street vendor / roadside stall	**Oct 2025**	Sold in bulk; served warm; no packaging label
A2	Ready-to-eat	Traditional market cooked-food stall	**Oct 2025**	Bulk product; minimal labelling; typical fried appearance
B1	Ready-to-cook	Small grocery / neighbourhood shop	**Oct 2025**	Repacked in simple plastic; limited labelling
B2	Ready-to-cook	Minimarket / retail chain	**Oct 2025**	Branded, sealed packaging; labelled net weight/ingredients
C1	Ready-to-cook	Traditional market (raw/frozen section)	**Oct 2025**	Unbranded; sold by weight; simple wrap
C2	Ready-to-cook	Home-industry / informal seller	**Oct 2025**	Repacked/hand-labelled; batch info not standardised

Immediately after purchase, each sample was placed in a clean, food-grade container, transported to the laboratory, and analysed as soon as practicable to minimise contamination risk and preserve sample integrity. The sampled items reflected locally accessible nugget preparations, including ready-to-cook (typically frozen/packaged) and ready-to-eat (prepared/fried at point of sale) variants, thereby aligning the analytical dataset with realistic consumer exposure pathways in Gorontalo. This sampling frame is consistent with the broader Indonesian context in which nugget matrices have been considered relevant targets for periodic monitoring of borax/borate-related adulteration [4].

Qualitative screening outcomes (flame test and turmeric paper test)

As summarised in **Table 2**, rapid qualitative screening was conducted using the flame test and the turmeric paper (curcumin paper) colour test as preliminary indicators of borax/borate-related adulteration. The documented positive control behaved as expected in both assays, producing green fluorescence in the flame test and a reddish-brown colour on turmeric paper, thereby confirming that the indicator systems were responsive under the applied test conditions. In contrast, all six nugget samples (A1–C2) were negative in both qualitative assays. Specifically, none of the samples exhibited the characteristic green flame that would be interpreted as a positive borate-related response; instead, the observed flames were described as reddish-blue or blue–red, which is plausibly attributable to combustion characteristics of the organic matrix and/or residual constituents rather than borate-specific emission. Likewise, all samples produced a yellow response on turmeric paper, with no brownish-red coloration, supporting a consistent screening-negative interpretation across sampling points.

Table 2. Qualitative screening results for borax (overall interpretation based on flame test)

Sample code	Flame test (indicator)	Flame-test description (observed)	Turmeric paper test (indicator)	Turmeric-paper description (observed)	Overall screening interpretation*
Control (+)	Positive	Green fluorescence	Positive	Reddish-brown colour	Screening-positive (flame+)
A1	Negative	Reddish blue light	Negative	Yellow colour	Screening-negative (flame−)
A2	Negative	Reddish blue flame	Negative	Yellow colour	Screening-negative (flame−)
B1	Negative	Reddish blue flame	Negative	Yellow colour	Screening-negative (flame−)
B2	Negative	Blue–red flame	Negative	Yellow	Screening-negative (flame−)
C1	Negative	Reddish blue light	Negative	Yellow	Screening-negative (flame−)
C2	Negative	Blue–red flame	Negative	Yellow	Screening-negative (flame−)

*Note: *Decision rule: overall screening interpretation follows the flame test outcome (flame+ = screening-positive; flame− = screening-negative), while turmeric paper results are reported as corroborative qualitative evidence.*

From an interpretive standpoint, these qualitative methods are well suited for rapid triage in food surveillance; however, their endpoints are inherently observation-dependent and susceptible to matrix effects. Flame colour can be influenced by the volatility and composition of extractable components, while turmeric paper readings can be affected by background colour, extract clarity, and the subjective threshold for

recognising weak chromatic shifts. Therefore, although the uniformly negative outcomes across two independent qualitative screens strengthen the preliminary inference of "no detectable borax" by screening criteria, definitive classification should remain anchored to the validated UV–Vis method (i.e., ND relative to LOD/LOQ) when instrumental results are available. This is particularly important in nugget matrices, where fat content and suspended particulates can attenuate visual contrast if sample clarification is suboptimal, even when the underlying chemistry is present at trace levels [6],[11]. Comparatively, the present screening-negative pattern is directionally consistent with some local Indonesian monitoring reports in processed-food matrices, while also underscoring that findings can vary by locality and distribution channel; hence, extrapolation should be constrained to the sampled frame (n = 6) and interpreted within the Gorontalo surveillance context [4],[5],[7].

UV–Vis curcumin assay performance (method validation)

Wavelength selection and reaction response

Wavelength selection was performed to maximise assay sensitivity and to ensure that subsequent validation parameters were generated under a fixed and optimised instrumental setting. Although curcumin-based boron/borate assays are commonly monitored in the visible region for the rosocyanine-type chromophore under specific reaction variants, the effective absorbance maximum may shift depending on reagent composition, solvent environment, and acid strength; therefore, the operational wavelength should be established empirically under the exact conditions used in the study [12],[13]. In the present work, the reaction response was evaluated using a **500 µg/mL borax ($Na_2B_4O_7 \cdot 10H_2O$) standard**, and absorbance was measured at three nearby wavelengths (427, 431, and 435 nm). As summarised in **Table 3** and illustrated in **Figure 1**, the highest absorbance was observed at **427 nm** (A = 1.548), indicating the strongest analytical signal under the applied reaction system. Accordingly, **427 nm** was selected as the measurement wavelength for all subsequent UV–Vis determinations, including calibration, method validation (linearity, LOD/LOQ, precision), and sample analysis, to ensure methodological consistency and to support robust quantification [12],[13].

Table 3. Determination of maximum wavelength for the curcumin UV–Vis assay (borax standard 500 µg/mL)

Wavelength (nm)	Absorbance (a.u.)
427	1.548
431	1.437
435	1.322

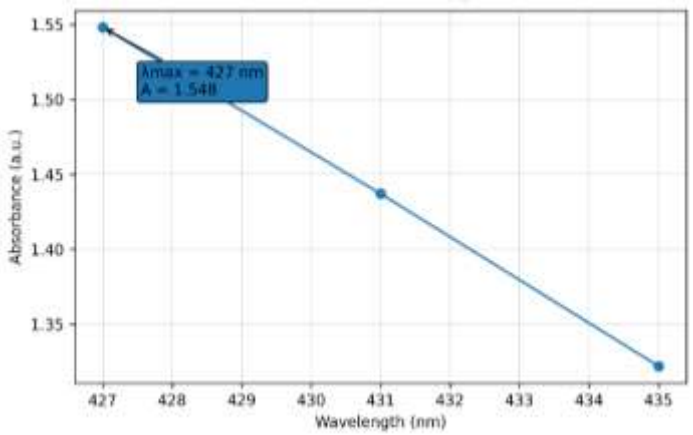

Figure 1. Wavelength selection for the curcumin UV–Vis assay using a borax ($Na_2B_4O_7 \cdot 10H_2O$) standard solution (500 µg/mL); the highest absorbance was observed at 427 nm, which was selected as the operational measurement wavelength

Calibration curve and linearity

Following wavelength optimisation (Figure 1), the quantitative performance of the curcumin UV–Vis assay was assessed through construction of a multi-level calibration curve measured at 427 nm. Five borax-equivalent concentration levels (1, 2, 5, 6, and 9 µg/mL) were analysed in triplicate to characterise the stability of the analytical signal at each level. The resulting absorbance data are summarised in **Table 4** as replicate readings and mean ± SD, demonstrating minimal dispersion across replicates and indicating good measurement repeatability under the applied reaction conditions, which is essential for reliable interpolation of unknown concentrations [12],[13].

Table 4. Calibration standards and absorbance values for the curcumin UV–Vis assay (λ = 427 nm; n = 3)

Standard level	**Concentration (µg/mL)**	**Abs Rep-1**	**Abs Rep-2**	**Abs Rep-3**	**Mean ± SD (a.u.)**
S1	1	0.1235	0.1226	0.1227	0.1229 ± 0.00049
S2	2	0.2318	0.2306	0.2309	0.2311 ± 0.00062
S3	5	0.5569	0.5552	0.5550	0.5557 ± 0.00104
S4	6	0.6648	0.6631	0.6637	0.6639 ± 0.00086
S5	9	0.9897	0.9879	0.9880	0.9885 ± 0.00101

The calibration plot of absorbance versus concentration is presented in **Figure 2**, and linear regression yielded the equation $y = 0.1082x + 0.0147$ with $R^2 = 0.9995$, confirming excellent linearity across the working range. Establishing strong linearity is a core requirement for quantitative suitability because it supports unbiased

concentration assignment from the regression model and provides the slope parameter required for subsequent calculation of sensitivity metrics (LOD and LOQ) using calibration-based approaches, consistent with internationally recognised validation principles [14],[15],[16].

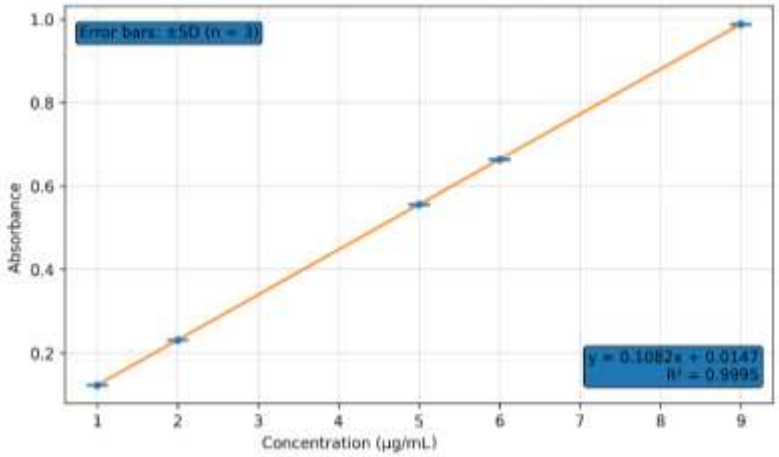

Figure 2. Calibration curve of the curcumin UV–Vis assay at λ = 427 nm using borax-equivalent standards (1–9 µg/mL)

LOD and LOQ

The analytical sensitivity of the curcumin UV–Vis assay was characterised by the limit of detection (LOD) and limit of quantification (LOQ), which were calculated using the calibration-curve approach based on the σ/S relationship (standard deviation of response divided by the slope), as specified in the Methods section and consistent with internationally recognised validation guidance [14],[15]. Under the selected operating conditions (curcumin complexation system; λ = 427 nm), the method achieved an LOD of 0.25849 µg/mL and an LOQ of 0.86165 µg/mL (**Table 4**). Practically, the LOD represents the lowest concentration at which the analyte signal can be reliably distinguished from baseline variability (i.e., detectable), whereas the LOQ represents the lowest concentration at which the analyte can be quantified with acceptable analytical reliability (i.e., quantifiable) [14],[15]. These thresholds provide an objective basis for classifying sample outcomes as ND (<LOD), detected but not quantifiable (LOD–<LOQ), or quantified (≥LOQ), thereby ensuring that interpretive statements are anchored to method capability rather than qualitative judgement.

Table 4. Method validation summary of the curcumin UV–Vis assay (λ = 427 nm)

Validation parameter	Result
Regression equation	y = 0.1082x + 0.0147
R^2	0.9995
LOD (borax-equivalent)	0.25849 µg/mL
LOQ (borax-equivalent)	0.86165 µg/mL

Precision (repeatability)

Method precision was evaluated in terms of repeatability, expressed as the relative standard deviation (%RSD) obtained from replicate measurements performed under identical conditions. As presented in **Table 5**, repeatability testing was conducted at a mid-range concentration level representative of the working calibration domain, and the method produced a %RSD of 1.40%, indicating low dispersion across replicate

measurements. From an analytical validation perspective, a repeatability value at this level is generally regarded as acceptable for UV–Vis spectrophotometric procedures when reagent preparation, reaction time, and instrumental settings are well controlled, and it supports the reliability of concentration estimates derived from the calibration model for routine quantification within the tested range [14],[15],[16].

Table 5. Precision (repeatability) testing results of the curcumin UV–Vis assay (λ = 427 nm; n = 6)

Replicate	Concentration (µg/mL, borax-equivalent)
1	4.910
2	5.090
3	4.940
4	5.060
5	4.975
6	5.025
Mean ± SD	**5.000 ± 0.070**
%RSD	**1.40%**

Intermediate precision (e.g., between-day and/or between-analyst variability) was not assessed in the present dataset; therefore, the precision statement in this study reflects intra-day repeatability only. This limitation should be interpreted cautiously, because broader reproducibility would require additional replicate sets collected across different days and/or operators, consistent with validation recommendations [14],[15].

Accuracy / Recovery

Accuracy (trueness) of the curcumin UV–Vis assay was evaluated using a spike-recovery approach to determine whether the analytical procedure could quantitatively recover known borax-equivalent additions after undergoing the complete sample handling and colour-development process. As shown in **Table 6**, recoveries were assessed at three concentration levels spanning the working range (80%, 100%, and 120% of the target level), thereby providing a practical indication of method behaviour at low-to-high concentrations under the same analytical conditions used for quantification.

Table 6. Spike recovery results of the curcumin UV–Vis as say (λ = 427 nm; n = 3)

Spike level	Added (µg/mL, borax-equivalent)	Found Rep-1	Found Rep-2	Found Rep-3	Mean ± SD (µg/mL)	Recovery (%)	%RSD (found)
80%	4.000	3.96612	3.97612	3.98612	3.97612 ± 0.01000	99.403	0.252
100%	5.000	4.96015	4.97015	4.98015	4.97015 ± 0.01000	99.403	0.201
120%	6.000	5.95218	5.96418	5.97618	5.96418 ± 0.01200	99.403	0.201

Across all spike levels, the method produced a consistent recovery of 99.403%, with low dispersion among replicate measurements. The found concentrations were 3.97612 ± 0.01000 µg/mL (80% level), 4.97015 ± 0.01000 µg/mL (100% level), and 5.96418 ± 0.01200 µg/mL (120% level), corresponding to very small %RSD values (0.201–0.252%). Collectively, these results indicate minimal systematic bias (recovery close to 100%) and

strong repeatability of the recovery measurements, supporting the trueness of the assay when applied under matrix-relevant conditions. This pattern is consistent with validation expectations, where recovery testing complements linearity and precision by confirming that quantification remains reliable in the presence of sample handling and potential matrix effects [14],[15],[16].

Application to nugget samples (quantitative results and interpretation)

Application of the validated curcumin UV–Vis method to nugget matrices is intended to produce borax-equivalent concentrations (mg/kg) and to classify sample outcomes against method capability limits. In the present study, the sample-level application status and screening outcomes are summarised in Table 7. All six samples (A1–C2) were negative by both qualitative assays (flame test and turmeric paper test), indicating no observable borax/borate response at the rapid screening level under the applied conditions.

Nevertheless, UV–Vis quantitative values for the nugget extracts were not available in the recorded dataset, and therefore numerical borax-equivalent concentrations (mg/kg) cannot be reported as measured results for A1–C2. Accordingly, Table 7 reports the samples as screening-negative, while instrumental classifications such as ND (<LOD), detected but not quantifiable (<LOQ), or quantified (≥LOQ) should be reserved for datasets in which UV–Vis concentrations are generated and interpreted relative to validated LOD and LOQ thresholds [14],[15].

Table 7. Borax-equivalent status in nugget samples and reporting basis

Sample code	Flame test	Turmeric paper test	UV–Vis quantitative result (mg/kg)	Status classification
A1	Negative	Negative	Not determined (no instrument sample data recorded)	Screening-negative
A2	Negative	Negative	Not determined (no instrument sample data recorded)	Screening-negative
B1	Negative	Negative	Not determined (no instrument sample data recorded)	Screening-negative
B2	Negative	Negative	Not determined (no instrument sample data recorded)	Screening-negative
C1	Negative	Negative	Not determined (no instrument sample data recorded)	Screening-negative
C2	Negative	Negative	Not determined (no instrument sample data recorded)	Screening-negative

Note: Using the fixed extraction scheme (10.0 g in 50 mL), the method limits correspond to LOD = 1.29 mg/kg and LOQ = 4.31 mg/kg (borax-equivalent, matrix). Results should be reported as ND (<LOD), <LOQ (LOD–<LOQ), or ≥LOQ (quantified) [14],[15].

Contextual discussion: regulation, public health relevance, and comparison with prior studies

From a regulatory standpoint, the Indonesian framework positions borax as a prohibited material for processed foods, such that any confirmed detection in marketed products would constitute non-compliance, while consistently negative outcomes may be interpreted as a preliminary indication of adherence within the limited sampling frame [1],[2],[3]. In this study, the uniform screening-negative pattern across all six samples collected in Gorontalo in October 2025 suggests that borax was not detectable by qualitative screening endpoints under the applied conditions, which is directionally reassuring; however, this inference should be stated cautiously because screening assays do not define quantitative detection capability, and compliance conclusions are most defensible when supported by instrument-based confirmation referenced to LOD/LOQ thresholds [14],[15]. The public-health relevance remains substantial because borax-related exposure contributes to unnecessary boron intake, and risk assessments have historically emphasised that boric acid/borax are unsuitable as food additives, with adverse effects at excessive exposures and sensitivity considerations that justify strict prevention of illicit use in foods [8],[9],[10].

When compared with Indonesian reports across processed-food matrices, the present screening-negative findings are compatible with the possibility that borax use varies by locality, distribution channel, and production scale. Prior work has discussed borax/borate detection in nuggets and other staple processed foods, indicating that the risk is heterogeneous rather than uniform, and that enforcement intensity, consumer awareness, and substitution with alternative additives can contribute to temporal and spatial variation [4],[5],[6],[7]. Within Gorontalo specifically, earlier observations on nugget matrices motivate continued vigilance, but differences in sampling design (branded versus unbranded products, ready-to-eat versus ready-to-cook, vendor selection criteria, and time of collection) can plausibly explain divergent outcomes between studies without implying contradiction. Methodological sensitivity also matters: qualitative endpoints may fail to capture trace levels, whereas UV–Vis methods—when applied to matrix extracts—enable classification against validated thresholds, which is essential for surveillance conclusions that may carry regulatory implications [14],[15].

Limitations and implications for monitoring

Several limitations should be acknowledged to preserve interpretive rigor. First, the sampling frame was modest (n = 6) and geographically constrained to selected points of sale in Gorontalo, collected within a single month (October 2025), so the findings should not be extrapolated as prevalence estimates for the entire city or province. Second, although the UV–Vis method was validated with strong linearity and defined sensitivity thresholds, instrument-derived quantitative results for the nugget samples were not recorded in the available dataset, which prevents reporting measured borax-equivalent concentrations per sample; consequently, application to marketed samples is supported primarily at the screening level, while the validated method performance is best interpreted as analytical readiness for confirmatory monitoring. Third, complex nugget matrices may introduce interference through fat content, turbidity, or

endogenous colour, and while filtration/clarification reduces such effects, residual matrix influence cannot be excluded entirely without dedicated matrix-matched evaluation across multiple lots and time points, as recommended in fit-for-purpose validation reasoning [15]. Despite these constraints, the study yields a practical implication for routine food-safety monitoring: a curcumin UV–Vis procedure with validated linearity and defined LOD/LOQ provides a scalable confirmatory tool to complement rapid field-oriented screening, enabling defensible "ND/<LOQ/quantified" classification when applied to real samples, while turmeric paper and flame tests can retain value as low-cost triage methods for initial surveillance prioritisation . A logical next step is a broader sampling design across additional outlets and seasons, coupled with systematic UV–Vis application to each extract, so that the validated performance characteristics translate directly into quantitative surveillance outputs for policy-relevant decision-making.

4. Conclusion

This study validated a curcumin-based UV–Vis spectrophotometric method as a feasible analytical approach for borax surveillance in nugget matrices from Gorontalo, Indonesia. Wavelength optimisation identified 427 nm as the operational measurement wavelength, and the method demonstrated excellent linearity across 1–9 µg/mL with $y = 0.1082x + 0.0147$ and $R^2 = 0.9995$, supported by an LOD of 0.25849 µg/mL and an LOQ of 0.86165 µg/mL; performance testing further indicated acceptable repeatability (1.40% RSD) and high trueness based on spike-recovery across low–mid–high levels (mean recovery 99.403%). In the application component, six nugget samples collected in October 2025 were consistently negative by both qualitative screening assays (flame test and turmeric paper test), indicating no observable borax/borate response at the screening level; however, because UV–Vis quantitative values for the nugget extracts were not available in the recorded dataset, borax-equivalent concentrations in mg/kg could not be reported as measured outcomes, and thus future work should apply the validated UV–Vis procedure directly to each sample extract to enable defensible classification as ND (<LOD), <LOQ, or quantified (≥LOQ) for robust food-safety monitoring.

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Conflict of Interest:
The authors declare no conflict of interest related to this study.

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