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Validation of an enzymatic method for glucose determination in potato juice (*Solanum tuberosum* L.) using Gluco-LIS®: Comprehensive evaluation according to ICH Q2(R1) and suitability for post-harvest quality control

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ABSTRACT

Controlling glucose content in potato tubers is a key challenge at two critical stages of the supply chain: (1) pre-harvest, to determine the optimal harvest date when glucose content is minimal and starch accumulation is maximal; (2) during post-harvest storage, to detect cold sweetening early and initiate preventive thermal reconditioning. This study reports the full validation of an enzymatic assay method based on the glucose oxidase–peroxidase (GOD/POD) system, implemented on the Gluco-LIS® portable reader, in accordance with ICH Q2(R1) and ISO 5725 requirements, conducted at the laboratory of the Technical Center for Potatoes and Artichokes (CTPTA, Manouba-Essaïda, Tunisia). Nine performance parameters were evaluated over the 10–500 mg/dL range: specificity, linearity, accuracy, repeatability, reproducibility, limit of detection (LOD), limit of quantification (LOQ), robustness, and stability. The calibration curve has $R^2 = 0.9999$ ($y = 1.0029x + 0.878$ mg/dL). Recovery rates range from 99.2% to 106.9%, repeatability CVs remain below 3.1% for c ≥ 100 mg/dL, and reproducibility CVs do not exceed 3.5% in a 3-day × 2-operator cross-validation. The operational LOQ was experimentally confirmed at 10 mg/dL (CV=9.9%) and covers the whole range of glucose levels in stored tubers. Robustness, tested by variation of laboratory ambient temperature (±2 °C), does not show a significant effect ($F = 3.158$; $p = 0.079$). The stability protocol has been adapted because of the intrinsic enzymatic instability of raw juice (browning with polyphenol oxidase – PPO), confirming the short-term chemical stability of D-glucose solution at 22 °C (H0-H3) and under refrigeration at 4 °C (J0-J3). These results validate Gluco-LIS® as a field tool for real-time post-harvest carbohydrate quality monitoring for potatoes following ICH Q2(R1).

Keywords: analytical validation, ICH Q2(R1), glucose oxidase, potato, Gluco-LIS®, reducing sugars, harvest date, cold sweetening, postharvest storage, CTPTA.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is an important part of global food systems. It is the fourth most important food crop in the world after wheat, maize and rice, with annual production of more than 375 million tons in over 150 countries (Devaux et al., 2021; Xu et al., 2023). The potato is the major vegetable crop in Tunisia in terms of area and production, and it plays an important role in food security and income of rural producers. The Spunta cultivar represents approximately 80% of the cultivated area thanks to its good storage and processing characteristics (Toumi, 2010).

Among the biochemical parameters determining the technological quality of the tuber, glucose content serves as a dual-purpose agronomic indicator: it reflects both the state of physiological maturity at harvest and the biochemical evolution of reducing sugars during post-harvest storage. During high-temperature cooking, glucose and fructose interact with the released amino acids in the Maillard reaction to form melanoidins that provide a dark brown color that is unacceptable

to consumers and acrylamide, a molecule classified as a potential carcinogen (Group 2A, IARC) (Gravoueille, 2004; Islam et al., 2022; Laumer et al., 2024). Industrial specifications generally set the maximum acceptable content of reducing sugars at 0.2–0.3% of fresh weight, or 111–167 mg/dL of tuber juice (Gravoueille, 2004).

IMPORTANCE OF MONITORING GLUCOSE CONTENT FOR DETERMINING THE OPTIMAL HARVEST DATE

The harvest date is one of the most critical agronomic factors for controlling the processing quality of potatoes intended for processing. Glucose, as an immediately available reducing sugar, is an indicator of the tuber's physiological maturity: its content changes inversely proportional to starch accumulation during the growing season.

Early in tuber growth, glucose content is high (potentially exceeding 200–300 mg/dL), reflecting the predominance of synthetic metabolic processes. During the maturation process, the sucrose→ starch pathway is enhanced by sucrose-phosphate synthase and ADP-glucose pyrophosphorylase (AGPase) leading to a gradual decrease in soluble sugars and an increase in dry matter and starch content (Datir et al., 2020; Cui et al., 2025). The glucose content at optimum harvest time is generally within the range of 30–80 mg/dL depending on the variety and climatic conditions (Sharkar et al., 2019; Krochmal-Marczak et al., 2020). Depending on the variety and climatic conditions, the glucose content at the optimum harvest time is usually in the range of 30–80 mg/dL (Sharkar et al., 2019; Krochmal-Marczak et al., 2020).

High glucose content at early harvesting promotes an intense Maillard reaction during frying and poor processing suitability. On the contrary, the late harvesting does not improve the biochemical quality significantly but increases the risk of mechanical damage to the tubers and fungal diseases. Regular monitoring of glucose levels during the growing season – which can be done in seconds in the field using a validated instrument such as the Gluco-LIS® – thus allows for an objective, analytically based decision regarding harvest timing, rather than relying on simple visual or calendar-based assessments. This approach, consistent with the principles of precision agriculture applied to the post-harvest phase, is directly applicable in the context of the CTPTA and Tunisian producer cooperatives.

IMPORTANCE OF MONITORING GLUCOSE PERCENTAGE DURING STORAGE

Post-harvest carbohydrate dynamics are governed by two opposing biological phenomena, the balance of which depends closely on storage temperature. The phenomenon of cold sweetening is triggered when the storage temperature drops below 8–10 °C: at low temperatures, the activity of phosphorylase, amylases, and acid invertase is not inhibited, leading to the gradual hydrolysis of starch into sucrose and then into glucose and fructose. Storage at 4 °C for less than two weeks may lead to the accumulation of reducing sugars to the level of 2–5% of the dry weight (Brążkiewicz et al., 2025; Krochmal-Marczak et al., 2020; Datir et al., 2020). Higher storage temperatures (>12–15 °C) accelerate cellular respiration, stimulate germination and phenolic degradation, which in turn, reduce the shelf-life and quality of the product (Abed et al., 2024; Pinhero and Yada, 2016).

On the contrary, higher storage temperatures (>12–15°C) accelerate cellular respiration, induce germination, and cause degradation of phenolics, thereby reducing the shelf life and quality of the product (Abed et al., 2024; Pinhero and Yada, 2016). The ideal storage period is between 8 °C and 12 °C for tubers destined for processing, a compromise between minimizing cold sweetening and postponing sprouting. Maintaining tubers within this window requires regular analytical monitoring of glucose levels.

Monitoring the glucose percentage during storage makes it possible to: (i) detect the onset of cold sweetening early, before the reducing sugar content reaches the technological threshold of unacceptability (0.25% PF, or ≈ 139 mg/dL); (ii) initiate thermal reconditioning in a timely manner (recond., 18–20 °C for 5–10 days), which allows for partial re-polymerization of sugars into starch and a return to glucose levels acceptable for processing (Pinhero and Yada, 2016); (iii) establish saccharification kinetic curves by batch and by variety, enabling the refinement of predictive preservation models. This ongoing analytical approach fully justifies the availability of a rapid, validated, and

inexpensive field tool such as the Gluco-LIS®, and constitutes the central application context of the present validation.

ANALYTICAL CONTEXT AND OBJECTIVE OF THIS STUDY

Reference methods for the quantification of sugars in plant matrices (HPLC-RI, HPLC-MS, automated spectrophotometry) require well-equipped laboratories, analysis time ranging from 30 to 60 min and trained personnel (Georgelis et al., 2018; Setyaningsih et al., 2021; Dini et al., 2023). These constraints make them unsuitable for monitoring in warehouse or raw material receiving settings. The Gluco-LIS®, a portable enzymatic reader based on the GOD/POD principle, delivers a result in less than one minute, without the need for laboratory infrastructure and at low operational cost (Navez et al., 2016; Gravoueille, 2004).

Although this instrument has long been used in Tunisian storage centers and processing units, no publication to date documents the systematic validation of its analytical performance against international standards. This gap constitutes an obstacle to the scientific credibility of the data produced and their acceptability in quality certification procedures. This paper aims to address this gap by reporting the first comprehensive validation of the Gluco-LIS® according to ICH Q2(R1) for the quantification of glucose in potato juice, and by establishing the link to two major application challenges: determining the optimal harvest date and monitoring post-harvest storage.

MATERIALS AND METHODS

Analytical principle

All analyses were performed at the laboratory of the Technical Center for Potatoes and Artichokes (CTPTA), located in Manouba-Essaïda, Tunisia (36°47'N, 10°02'E). The Gluco-LIS® is a portable photometric reader designed for the rapid analysis of glucose on test strips. The analytical reaction is based on a two-step GOD/POD enzymatic coupling:

- Glucose oxidase (EC 1.1.3.4) selectively oxidizes D-glucose in the β-configuration to D-gluconic acid, with stoichiometric production of hydrogen peroxide (H_2O_2);
- Peroxidase (EC 1.11.1.7) catalyzes the reduction of H_2O_2 in the presence of a chromogen, generating a colorimetric signal proportional to the glucose concentration, displayed in mg/dL after a 30-second reading in the range of 10–600 mg/dL (Nakamura and Ogura, 1968; WHO, 2006). Figure 1(a) shows the general view of the CTPTA analytical workstation; Figure 1(b) illustrates a measurement session with standard solutions applied to the Gluco-LIS® test strips.

Reagents and reference materials

The primary reference substance was pure anhydrous D-glucose (≥ 99%, analytical grade). A 1.000 mg/dL stock solution was prepared each day of analysis by accurately dissolving 10 g in 100 mL of distilled water. Working solutions at concentrations of 10, 50, 100, 200, 300, and 500 mg/dL

Figure 1. Experimental setup at the CTPTA laboratory, Manouba-Essaïda, Tunisia.
(a) General view of the workstation with the Gluco-LIS®, the RADWAG AS 220.R2 analytical balance, analytical reagents, and Spunta test tubes; (b) Measurement session with standard solutions and test strips

were obtained by precise successive dilutions according to the relationship $C_1V_1 = C_2V_2$ (Table 1).

For interference studies (Figure 2), pure D-fructose (≥ 99%), sucrose, and ascorbic acid were prepared at the same concentrations and added to the corresponding glucose solutions. The matrix blank was distilled water (conductivity < 1 µS/cm). All reagents were used without further purification. Table 2 presents the complete technical specifications for each reagent used, taken from the suppliers' technical data sheets. Figure 2(a) shows the reagent bottles used for interference studies; Figure 2(b) illustrates the gravimetric preparation of the 1.000 mg/dL primary stock solution on the RADWAG AS 220.R2 analytical balance (d = 0.1 mg).

Important note: The D-fructose used (SRL, cat. no. 42868) contains up to 0.5% residual D-glucose according to the technical data sheet. Far from being a limitation, this fact reinforces the scientific value of the specificity results: despite this residual glucose contamination, no significant interference was detected, confirming the robustness of the two-tailed t-test used. Ascorbic acid was used in its sodium salt form (sodium L-ascorbate, CAS 134-03-2), which is less reactive than the free acid with respect to the peroxidase system.

Preparation and extraction of potato juice

The plant material was derived from five tubers representative of the Spunta variety (*Solanum tuberosum* L.), sourced from the same harvest batch and stored under standardized conditions (temperature: 7 ± 1 °C; relative humidity: 88 ± 2%). After washing under running water and standardized manual peeling, the tubers were mechanically ground until a homogeneous paste was obtained. Juice extraction was performed by centrifugation (Figure 3(b)) at 3,000 rpm for 10 minutes. The supernatant was filtered through a fine-mesh membrane (Figure 3(c)) to remove residual particles, then precisely diluted to fall within the instrument's linearity range before being applied to the test strip (Gravoueille, 2004). Figure 3(a) shows the extraction equipment used; Figure 3(b) illustrates the centrifugation step for potato juice extraction at 3.000 rpm for 10 minutes; Figure 3(c) shows the filtration of the extract through a fine-mesh membrane prior to analysis.

Validation experimental design according to ICH Q2(R1)

Validation was conducted in accordance with ICH Q2(R1) (EMA, 2005) and SFSTP recommendations (2003), in accordance with practices documented in recent analytical validation literature (Shinde and Khulbe, 2025), rigorously following the standard sequence: specificity → linearity → accuracy → precision → LOD/LOQ → robustness → stability. For each parameter, the normality of the residuals was tested using the Shapiro-Wilk test (Shapiro and Wilk, 1965), the homogeneity of variances using the Cochran test (calculated C vs. tabulated C at 5% and 1%), and between-group comparisons using one-way ANOVA combined with the Kruskal-Wallis test ($\alpha = 0.05$).

Specificity, linearity, accuracy, precision, LOD/LOQ

These five parameters were evaluated on pure D-glucose solutions according to the following protocols: (i) specificity using a two-tailed Student's t-test ($\alpha = 5\%$, ddl = 8) comparing measurements of pure glucose to measurements in the presence of each interferent (fructose, sucrose, ascorbic acid) at three concentration levels, for a total of nine comparisons; (ii) linearity using

Table 1. Preparation of standard solutions by dilution of the stock solution (1.000 mg/dL)

Concentration (mg/dL)	Stock V (mL)	V water (mL)	Final V (mL)	Justification
0 (negative control)	0	100	100	Background noise check
10	0.10	99.90	100	Near LOQ — low-range sensitivity
50	0.50	99.50	100	Low-range zone
100	1.00	99.00	100	Midpoint — accuracy
200	2.00	98.00	100	Upper linearity range
300	3.00	97.00	100	Optimal upper limit
500	5.00	95.00	100	Out-of-range test — extended linearity

Table 2. Technical specifications of the reagents used — supplier data (SRL and SUVCHEM)

Reagent	Grade	CAS	MW (g/mol)	Purity / Assay	Supplier / Ref.	Key feature
D-Glucose (Dextrose)	Extrapure	50-99-7	180.16	Extrapure	SRL / 42738	[α] = +52.0° to +53.0°; loss on drying ≤ 1%
D-Fructose	Extrapure	57-48-7	180.16	≥ 98% (HPLC)	SRL / 42868	[α] = −91° to −93°; residual D-glucose ≤ 0.5%
Sodium L-ascorbate	Extrapure	134-03-2	198.11	≥ 99% (iodometric)	SRL / 65265	Sodium salt; [α] = +103° to +108°
Sucrose	LR	57-50-1	342.3	90–100%	SUVCHEM / S0198700500	pH neutral in solution; standard stability

Figure 2. Analytical reagents and precision weighing. (a) bottles of saccharose LR (SUVCHEM) and extra-pure D-glucose (SRL ref. 42738) with Spunta stoppers; (b) weighing of 1.0002 g of D-glucose on a RADWAG AS 220.R2 analytical balance (accuracy d = 0.1 mg) for preparation of the 1.000 mg/dL stock solution

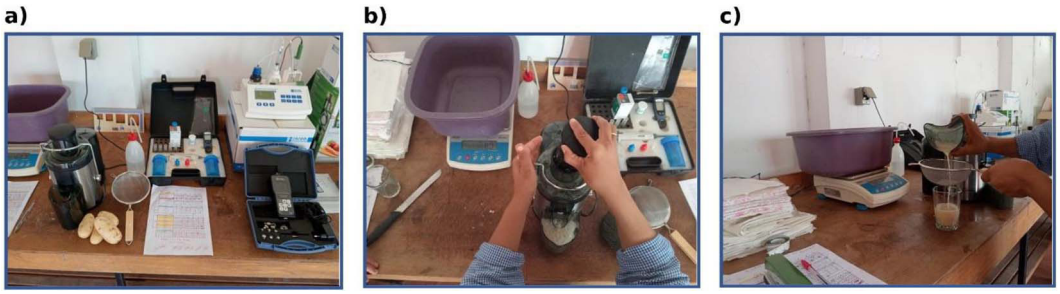

Figure 3. (a) Juice extraction equipment (centrifuge, fine-mesh membrane, Gluco-LIS), (b) potato juice extraction, (c) juice filtration using a fine-mesh membrane

OLS regression on N = 30 measurements (6 levels × 5 replicates) with an F-test for lack of fit, Cochran's test, and Shapiro-Wilk test on the residuals; (iii) accuracy by calculating the recovery percentage (%R = x_found / x_theoretical × 100) and relative bias at the six levels; (iv) intra-day repeatability (n = 6, 3 levels) and reproducibility according to a 3-day × 2-operator × 3-replication cross-over design (N = 54 measurements); (v) LOD and LOQ using three complementary approaches: technical limit, regression analysis, and experimental validation.

Robustness

The robustness of the method was evaluated according to the Youden and Steiner (1975) procedure, adapted for field devices in accordance with SFSTP (2003) recommendations.

The operational parameter tested is the ambient temperature of the laboratory at the time of the enzymatic reaction on the test strip. This parameter is the variable most likely to fluctuate unintentionally under actual conditions of use at the CTPTA (Manouba-Essaïda, Tunisia), where the

indoor laboratory temperature typically varies between 24 °C and 28 °C depending on the season and ventilation conditions. According to Arrhenius' law, the enzymatic activity of glucose oxidase (EC 1.1.3.4) and peroxidase (EC 1.11.1.7) is temperature-sensitive; a fluctuation of ±2 °C within the 24–28 °C range is likely to induce slight variations in the analytical signal, on the order of 3–5% according to data in the literature (Tang et al., 2000; Nakamura and Ogura, 1968).

Three independent conditions were tested around the nominal temperature of 26 °C, representative of actual conditions in the CTPTA laboratory, using a freshly prepared 100 mg/dL solution of pure D-glucose (n = 5 independent measurements per condition, N = 15 measurements in total). The ambient temperature was verified using a calibrated thermometer (accuracy ±0.5 °C) after 30 minutes of complete thermal stabilization. The test strips and solutions were placed in the laboratory 30 minutes before the start of the measurements to ensure thermal equilibration. Acceptance criterion: one-way ANOVA, $p > 0.05$ → robust method (Table 3).

Stability of the analyte

During preliminary experiments at the CTPTA, storing raw potato juice at 4–5 °C for 24–72 hours resulted in intense darkening of the solution accompanied by a pungent (sulfur-fermented) odor, rendering the sample unsuitable for any colorimetric analysis using the Gluco-LIS®. This phenomenon corresponds to enzymatic browning mediated by polyphenol oxidase (PPO, EC 1.10.3.1), released during tuber grinding, which oxidizes endogenous phenolic compounds – primarily chlorogenic acid and caffeic acid, whose levels in Spunta juice range from 80 to 450 mg/kg fresh weight depending on genotype and growing conditions (Genovese et al., 2019; Matheis and Whitaker, 1984). At 4 °C, PPO retains 30–50% of its maximum activity and induces visible browning as early as Day 1.

Consequently, two complementary stability protocols were implemented (Table 4). The first assessed short-term stability on a laboratory bench (22 ± 1 °C), representative of the actual time interval between extraction and analysis under field conditions. The second assessed refrigerated stability (4 ± 1 °C), simulating inter-session storage. Both protocols were applied to pure D-glucose solutions (Step 1) and to enriched potato juice (Step 2, limited prior to enzymatic browning).

RESULTS AND DISCUSSION

Specificity

Specificity was evaluated by comparing measurements of pure glucose to measurements in the presence of each of the three potential interferents (fructose, sucrose, and ascorbic acid) at three concentration levels. Nine two-tailed Student's t-tests were performed ($\alpha = 5\%$, df = 8, t_crit = 2.306). Table 5 presents the complete Student t-test results for all nine interferent–concentration comparisons. Figure 4(a) shows the two-tailed Student's t-test results for the nine interferent–concentration comparisons, confirming specificity for all tested conditions.

None of the nine comparisons reaches the significance threshold: |calculated t| ranges from 0.27 to 1.55, all significantly lower than t_crit = 2.306. These results can be explained by the high structural specificity of glucose oxidase for β-D-glucopyranose at the C1 position (Km for fructose > 10 mol/L vs. Km for glucose = 33–110 mmol/L; Nakamura and Ogura, 1968), and by the fact that the ascorbic acid concentrations tested remain below the peroxidase system's interference threshold (> 500 mg/dL; Tang et al., 2000). The Gluco-LIS® method is therefore selective for D-glucose under the matrix conditions of potato juice.

Table 3. Experimental design for the robustness test – Pure D-glucose 100 mg/dL – 3 conditions × n = 5

Condition	Lab temperature (°C)	Solution analyzed	n	Justification
1 — Nominal	26 ± 0.3	Pure D-glucose 100 mg/dL	5	Actual nominal temperature of the CTPTA laboratory
2 — Low temperature	24 ± 0.3	Pure D-glucose 100 mg/dL	5	Active air conditioning (T − 2 °C)
3 — High temperature	28 ± 0.3	Pure D-glucose 100 mg/dL	5	Active climate control (T + 2 °C)

Table 4. Complete experimental design of the stability test – ICH Q2(R1) – two alternatives × two steps

Step	Alternative	Solution	T° (°C)	Test periods	n/time	Acceptance criterion
STEP 1 — Pure analyte	A — Short-term (bench)	Pure D-glucose 100 mg/dL	22 ± 1	H0, H1, H2, H3	5	Variation < 5% vs. H0; ANOVA p > 0.05
	B — Refrigeration	Pure D-glucose 100 mg/dL	4 ± 1	Day 0, Day 1, Day 2, Day 3	5	Variation < 5% vs. Day 0; ANOVA p > 0.05
STEP 2 — Juice matrix	B — Refrigeration (limited before browning)	Enriched potato juice (+ 100 mg/dL glucose added)	4 ± 1	D0, D1, D2, D3	5	Variation < 5%; stop if browning occurs

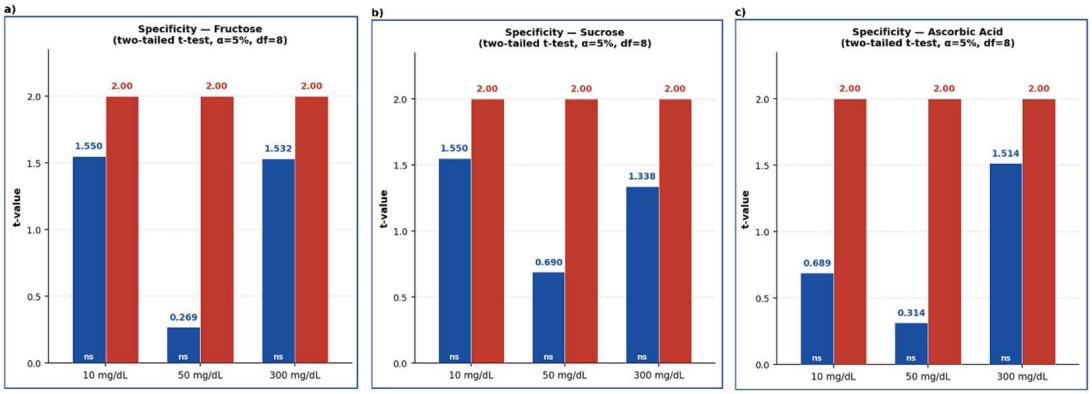

Figure 4. Specificity of the Gluco-LIS® method – (a) two-tailed Student's t-tests for all nine interferent – concentration comparisons (3 interferents × 3 concentration levels). |t_calculated| ranges from 0.27 to 1.55, all below t_crit = 2.306 (df = 8, α = 5%): no significant interference detected

Table 5. Specificity / Selectivity — Two-tailed t-tests (α = 5%, df = 8, t_crit = 2.306) — 3 interferents × 3 levels

Interferent	C (mg/dL)	Mean Pure Glu	Mean Glu+Int.	Diff.	|t| calc.	p-value	Decision
Fructose	10	12.13	11.53	0.60	1.550	0.1324	✓ ns
Fructose	50	51.15	51.00	0.15	0.269	0.7898	✓ ns
Fructose	300	301.83	301.00	0.83	1.532	0.1380	✓ ns
Sucrose	10	12.13	11.53	0.60	1.550	0.1324	✓ ns
Sucrose	50	51.15	50.80	0.35	0.690	0.4957	✓ ns
Sucrose	300	301.83	301.06	0.78	1.338	0.1913	✓ ns
Ascorbic acid	10	12.13	11.87	0.27	0.689	0.4966	✓ ns
Ascorbic acid	50	51.15	51.00	0.15	0.314	0.7560	✓ ns
Ascorbic acid	300	301.83	300.94	0.89	1.514	0.1405	✓ ns

Linearity

Linearity was evaluated at six concentrations (10, 50, 100, 200, 300, and 500 mg/dL) with five replicates per level (N = 30 measurements). The linear regression of measured concentrations against theoretical concentrations was calculated using the ordinary least squares (OLS) method. Homogeneity of variances was tested using the Cochran test, and the linearity of the model was tested using the F-test for lack of fit. Table 6 summarises the regression parameters and statistical tests for all 30 measurements. Figure 6 presents the three statistical diagnostics of the OLS residuals: Figure 6(a) shows the QQ-plot confirming normality (Shapiro-Wilk: W = 0.9745, p = 0.667 > 0.05); Figure 6(b) presents the residual frequency distribution; Figure 6(c) shows the residuals vs. fitted values plot confirming homoscedasticity.

The coefficient of determination $R^2 = 0.9999$ is far above the minimum required level of 0.99 as recommended by ICH Q2(R1). The slope a = 1.0029 is well within the acceptance interval

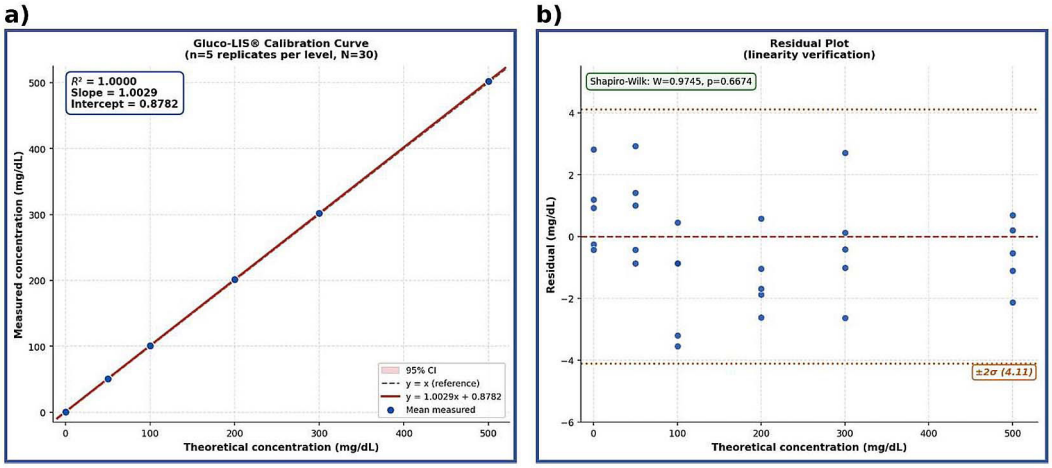

Figure 5. Gluco-LIS® linearity – (a) Calibration curve (y = 1.0029x + 0.878 mg/dL; R^2 = 0.9999; N = 30; 95% CI shown as red hatched lines); (b) Residual plot confirming the absence of systematic trend and homoscedasticity of the OLS model.

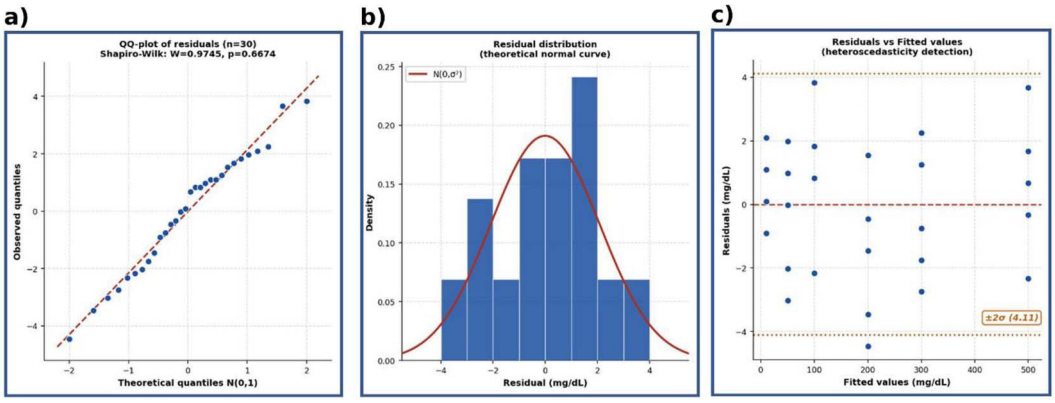

Figure 6. Statistical diagnostics of the OLS residuals – (a) QQ-plot confirming normality (Shapiro-Wilk: W = 0.9745, p = 0.667); (b) residual frequency distribution; (c) residuals vs. fitted values plot confirming homoscedasticity.

[0.95; 1.05], indicating a concentration proportional response, with no significant amplification/attenuation factors.

The intercept b = 0.878 mg/dL, though below the critical threshold of 5 mg/dL, is not statistically different from zero ($|t| = 1.482 < t_{tab} = 2.048$, ddl = 28, α = 5%), confirming the absence of significant systematic bias at the origin. This result indicates that the calibration response is proportional to the glucose concentration, without detectable baseline offset within measurement uncertainty across the validated range (10–500 mg/dL).

The homogeneity of variances is confirmed by the Cochran test ($C = 0.2253 < C5\% = 0.4564$), validating the OLS regression. The F-test for lack of fit ($F_{lof} = 1.496 < F_{tab} = 2.776$) confirms the adequacy of the linear model on the whole range studied.

The performance metrics are comparable to ISO 15197-certified glucose meters (R^2 = 0.997–0.999; Freckmann et al., 2012) and to HPLC methods validated on plant extracts (Setyaningsih et al., 2021).

Linearity of the Gluco-LIS® method: mechanistic and physicochemical justification

The calibration curve established over the 10–500 mg/dL range yields a coefficient of determination R^2 = 0.9999 (slope a = 1.0029 ± 0.003; intercept b = 0.878 mg/dL), confirmed by the lack-of-fit test ($F_{LOF} = 1.496 < F_{tabulated} = 2.776$, α = 5%, df = 4 and 24) and homogeneity of residual variances (Cochran test: $C = 0.225 < C_5\% = 0.456$) (Figure 5). Figure 5(a) shows the calibration curve with the 95% confidence interval; Figure 5(b) presents the residual plot confirming the absence of systematic trend. This

Table 6. Linearity – regression parameters and statistical tests – N = 30 measurements (6 levels × 5 replicates)

Parameter	Value obtained	ICH Q2(R1) criterion	Status
Regression equation	y = 1.0029x + 0.8782	y = 1.0000x + 0	—
R^2 coefficient	0.9999	≥ 0.99	✓ CONFORMS
Slope (a)	1.0029	0.95 – 1.05	✓ COMPLIANT
Intercept (b)	0.878 mg/dL	|b| ≤ 5 mg/dL	✓ CONFORMS
Shapiro-Wilk (residuals)	W = 0.9745; p = 0.667	p > 0.05	✓ Normality validated
Cochran test (variances)	C = 0.2253 < C_5% = 0.4564	C < tabulated C	✓ Homogeneous variances
F-statistic for lack of fit	F_lof = 1.496 < F_tab = 2.776	F < F tabulated	✓ Linearity confirmed
t-test (intercept ≠ 0)	|t| = 1.482 < t_tab = 2.048	|t| < t tabulated	✓ Residual bias ns

level of linearity finds direct explanation in the kinetics of the GOD/POD enzymatic reaction. The reaction rate catalysed by glucose oxidase (GOD, EC 1.1.3.4) follows the Michaelis-Menten model (Equation 1):

$$v = Vmax \times [S] / (Km + [S]) \quad (1)$$

where: [*S*] denotes the D-glucose substrate concentration and *Km* the Michaelis constant.

For glucose oxidase from Aspergillus niger – the enzyme used in virtually all commercial GOD/POD reagents, including Gluco-LIS® – Gibson et al. (1964) determined Km ≈ 33 mM for β-D-glucose in aqueous medium. Leskovac et al. (2005) confirmed this value in a mechanistic study of the GOD active site. The maximum concentration of the working range adopted in this study is 500 mg/dL = 27.75 mM, i.e. [*S*]*max* / *Km* ≈ 0.84. In this regime, the theoretical deviation from linearity with respect to the first-order law ($v \approx (Vmax/Km) \times [S]$) remains below 4%, a value that is moreover compensated by the linear response of the peroxidase-chromogen detection step (POD, EC 1.11.1.7), whose substrate H_2O_2 is produced in stoichiometric quantity and lies below enzymatic saturation over this range. The entire GOD/POD system therefore operates in a quasi-linear first-order kinetic regime over 10–500 mg/dL, providing the mechanistic basis for the R^2 observed.

The signal measured by the Gluco-LIS® reader is a photometric absorbance at 505–546 nm of the coloured product formed during the POD-chromogen reaction. This absorbance follows Beer-Lambert's law ($A = \varepsilon \times l \times C$), a strictly linear relationship between absorbance and concentration for C < 0.01 mol/L and A < 2.0 AU – conditions fully satisfied over the 10–500 mg/dL range on pure D-glucose standards (≥ 99%, Sigma-Aldrich) dissolved in distilled water (conductivity < 1 µS/cm), constituting a single-component, interference-free matrix (Lott and Turner, 1975). The combination of the quasi-linear GOD/POD kinetic regime and Beer-Lambert's law mechanistically yields an R^2 very close to unity under these controlled experimental conditions.

Bibliographic positioning and concordance with published methods

This analytical performance is consistent with all published data for GOD/POD enzymatic methods validated on glucose reference standards (Table 7). Mora-Marín et al. (2021), in a complete GOD-PAP method validation applied to human sera, report a Pearson correlation coefficient r = 0.9988, a repeatability CV of 2.1%, an intermediate precision CV of 1.9% and a recovery of 99.15%. Bhatt et al. (2021) confirm the linearity of the end-point GOD/POD method up to 500 mg/dL with r = 0.99 on human plasma, identical to the result obtained in the present study. Pereira et al. (2008), who validated a GOD/POD enzymatic method by UV-Vis spectrophotometry directly on Solanum tuberosum tuber matrix following the INMETRO DOQ-CGCRE-008 guide, obtained a mean recovery of 99.0% and linearity from 1.25 to 40 µg, confirming the transferability of this system's performance to the vegetable matrix concerned.

The distinction between validation on pure standards and evaluation on real matrix warrants emphasis. These two evaluation levels are complementary under ICH Q2(R1): linearity on pure standards characterises the intrinsic analytical performance of the measurement system under ideal conditions, while accuracy on spiked natural potato juice (recovery 99.2–106.9%, n = 30) (Figure 7) demonstrates fitness for purpose in the

Table 7. GOD/POD linearity parameters reported in the literature – comparison with the Gluco-LIS® method

Reference	Matrix	Range (mg/dL)	r or R^2	CV (%)	Recovery (%)
Trinder (1969)	Human serum	0–500	$R^2 \approx 1$	N.R.	N.R.
Lott and Turner (1975)	Serum / Urine	0–500	$R^2 \approx 0.999$	< 3.5	N.R.
Bergmeyer (1983)	Clinical standardisation	0–500	$R^2 \approx 0.999$	< 3.0	N.R.
Pereira et al. (2008)	*S. tuberosum* tuber	1.25–40 µg	Linear	< 4.5	99.0
Mora-Marín et al. (2021)	Human sera	N.R.	r = 0.9988	2.1 / 1.9	99.15
Bhatt et al. (2021)	Human plasma	0–500	r = 0.99	N.R.	N.R.
Present study — Gluco-LIS®	Juice *S. tuberosum* var. *Spunta*	10–500	R^2 = 0.9999	< 3.5	99.2–106.9

Note: N.R. – not reported. CV: intra-day repeatability. Sources: Trinder (1969); Lott and Turner (1975); Bergmeyer (1983); Pereira et al. (2008); Mora-Marín et al. (2021); Bhatt et al. (2021).

real biological matrix, in the presence of all potentially interfering plant constituents (polyphenols, organic acids, proteins, residual starch). The concordance between these two performance levels – attested by recovery rates close to 100% – confirms that the quasi-perfect linearity observed on pure standards translates into effective analytical accuracy on real samples.

Accuracy

Accuracy was evaluated by calculating the recovery percentage (%R = x_found / x_theoretical × 100) and the relative bias (Bias% = (x_found − x_theoretical) / x_theoretical × 100) for the six concentration levels. The recovered concentrations were calculated using the calibration equation: x = (y − 0.8782) / 1.0029. Figure 7(a) shows the % recovery at each concentration level; Figure 7(b) presents the corresponding % bias values, both within the ICH Q2(R1) acceptance criteria.

Recovery ranges from 99.16% to 106.91%, meeting the ICH Q2(R1) criteria of 95–115% for all concentrations. The maximum bias of +6.91% at 10 mg/dL is expected near the LOQ (a universal phenomenon in enzymatic methods predicted by the Horwitz equation). For concentrations ≥ 50 mg/dL – the primary post-harvest application range – recovery stabilizes in the excellent range of 99.2–102.0%.

Repeatability (intra-day precision)

Repeatability was assessed using six consecutive measurements (n = 6) performed on the same day by the same operator using the same instruments and reagents for three concentration levels (30, 100, and 500 mg/dL). The coefficient of variation (CV%) and the

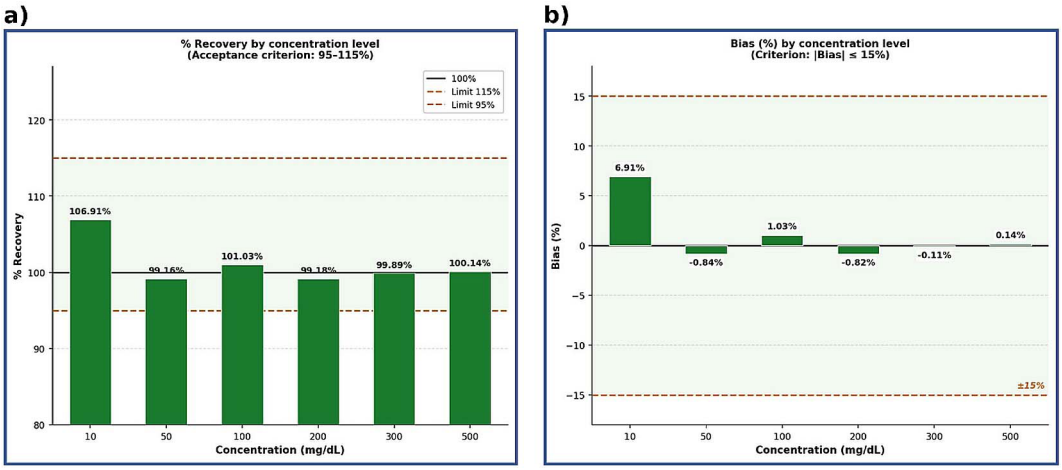

Figure 7. Accuracy of the Gluco-LIS® method – (a) % recovery by concentration level (target: 95–115%, ICH Q2(R1)); (b) % bias by concentration level (target: |bias| ≤ 15%). Green areas = ICH Q2(R1) acceptance zones

Table 8. Accuracy – % recovery, relative bias – 6 levels × 5 replicates

Theoretical C (mg/dL)	n	Mean (mg/dL)	Retro. mean (mg/dL)	SE	CV (%)	% Recoverability	Bias (%)	Status
10	5	11.60	10.69	1.137	10.63	106.91	+6.91	✓
50	5	50.60	49.58	2.068	4.17	99.16	−0.84	✓
100	5	102.20	101.03	2.162	2.14	101.03	+1.03	✓
200	5	199.80	198.35	2.381	1.20	99.18	−0.82	✓
300	5	301.40	299.66	2.068	0.69	99.89	−0.11	✓
500	5	503.00	500.68	2.230	0.45	100.14	+0.14	✓

repeatability coefficient (Cr) (= 2.77 × Sr, ISO 5725-2) were calculated.

The repeatability CVs (Figure 8) are 0.72% (500 mg/dL), 3.10% (100 mg/dL), and 6.34% (30 mg/dL), meeting the ICH Q2(R1) criteria of CV < 5% for concentrations ≥ 100 mg/dL and CV < 15% for low concentrations (30 mg/dL). The decrease in CV with concentration is expected and consistent with the behavior of enzymatic methods. The repeatability coefficients Cr (5.18–9.91 mg/dL) quantify precision in absolute terms (Table 8). Table 9 provides the complete repeatability statistics including the Cochran homogeneity test.

Reproducibility (intermediate precision)

Reproducibility (inter-laboratory precision) was evaluated using a 3-concentration × 3-day × 2-operator × 3-replication crossover design (18 groups, N = 54 measurements). A one-way ANOVA was used to distinguish between between-group and within-group variance, yielding the reproducibility CV. The detailed ANOVA statistical results for the three concentration levels are compiled in Table 10. Figure 9 illustrates the inter-day/inter-operator reproducibility results: Figure 9(a) shows the CV% profiles for the three concentration levels; Figure 9(b) compares the calculated F values against the tabulated F threshold (F_tab = 3.106), confirming the absence of significant day or operator effect.

The reproducibility CVs (0.55–3.50%) largely satisfy the ICH Q2(R1) criterion of CV < 10%. The ANOVA detects no significant effect of the day/operator factor (F = 0.296–0.633 < F_(tabled) = 3.106; p = 0.68–0.91), confirming the absence of inter-session bias. The Gluco-LIS® method is robust against the inevitable variations from one session to another. These performance results are superior to the data reported by Dini et al. (2023) for automated GOD methods and validate the Gluco-LIS® for multi-operator deployment under industrial conditions.

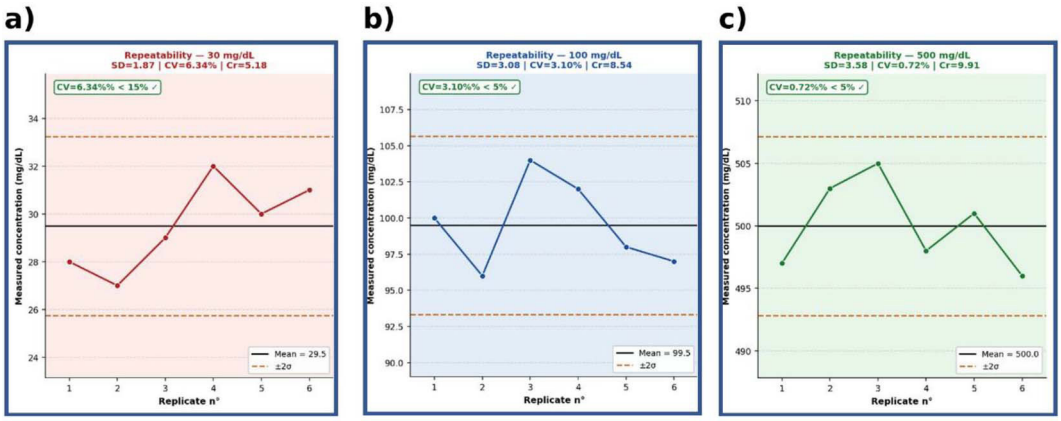

Figure 8. Intra-day repeatability of the Gluco-LIS® method – (a) Individual measurements at three concentration levels (30, 100, and 500 mg/dL; n = 6 each). Black line = mean; orange band = ±2 SD. CV < 5% for c ≥ 100 mg/dL; CV < 15% for 30 mg/dL

Table 9. Repeatability – complete statistics – 3 levels × 6 replicates – Cochran test

Concentration	n	Mean (mg/dL)	SE (Sr)	CV%	Cr (2.77·Sr)	CV criterion	Status
30 mg/dL	6	29.50	1.8708	6.34%	5.1822	< 15%	WITHIN NORM
100 mg/dL	6	99.50	3.0822	3.10%	8.5377	< 5%	COMPLIANT
500 mg/dL	6	500.00	3.5777	0.72%	9.9103	< 5%	COMPLIANT

Note: Cochran's test: C = 0.4961 < C_5% = 0.7069 (k=3, n=6) → homogeneity of variances ✓.

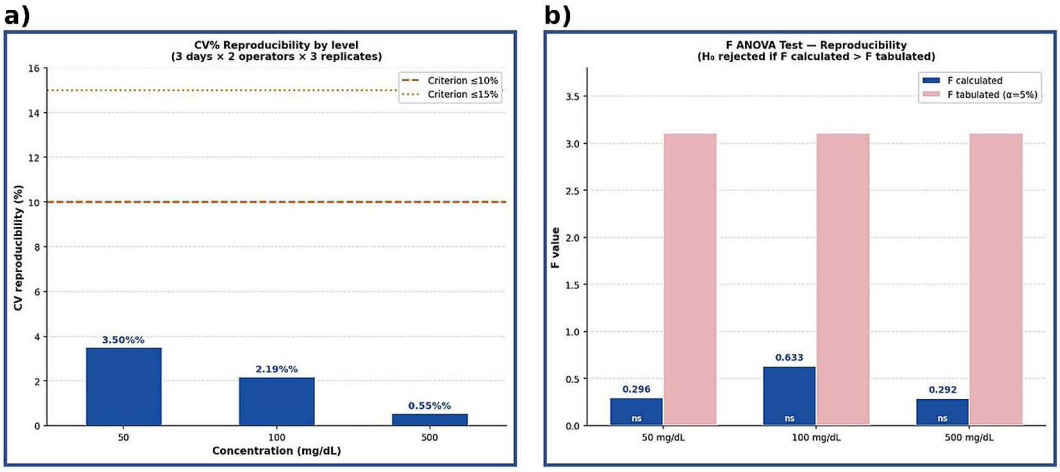

Figure 9. Reproducibility of the Gluco-LIS® method — (a) CV% profiles for the three concentration levels across days and operators (3-day × 2-operator crossover design); (b) comparison of calculated F values vs. tabulated F threshold (F_tab = 3.106, α = 5%): F_calc << F_tab for all levels, confirming the absence of significant day or operator effect

Table 10. Reproducibility – one-way ANOVA – statistical results – 3 levels

Concentration	N	Groups k	Calculated F	Tabled F	p ANOVA	Sig.	CV%	Status
50 mg/dL	18	6	F=0.296	F_tab=3.106	p = 0.9058	ns	CV=3.50%	CONFORMS
100 mg/dL	18	6	F=0.633	F_tab=3.106	p = 0.6788	ns	CV=2.19%	CONFORMS
500 mg/dL	18	6	F=0.292	F_tab=3.106	p = 0.9084	ns	CV=0.55%	CONFORMS

Note: Cochran test (k=18, n=3): C 0.1022 < C_5% = 0.293 for all levels → homogeneity of variances confirmed under all conditions (3 days × 2 operators).

Limits of detection (LOD) and quantification (LOQ)

Three complementary approaches were used to determine the LOD and LOQ, in accordance with ICH Q2(R1) recommendations:

- Method 1 — Technical limit of the instrument: the matrix blank (distilled water) consistently measures L0 (< 10 mg/dL not displayed) → Technical LOD = 10 mg/dL
- Method 2 — Regression-based: LOD = 3.3 × Se/a = 6.99 mg/dL; LOQ = 10 × Se/a = 21.19 mg/dL
- Method 3 — Experimental validation: 10 measurements at 10 mg/dL → mean = 11.70 mg/dL, CV = 9.91% ≤ 20% A comparative synthesis of the three LOD/LOQ estimation approaches is provided in Table 11. Figure 10 illustrates the LOD/LOQ determination: Figure 10(a) shows the 10 replicate measurements at 10 mg/dL used to experimentally confirm the LOQ (CV = 9.91% ≤ 20%); Figure 10(b) compares the three calculation approaches (technical limit, OLS-regression, and experimental), all converging to LOQ = 10 mg/dL

The convergence of the three approaches toward an LOQ of 10 mg/dL reinforces the consistency and validity of this threshold. According to the data of Gravoueille (2004), Burton (1989) and Galdón et al. (2010), glucose concentrations in the context of the intended application are generally

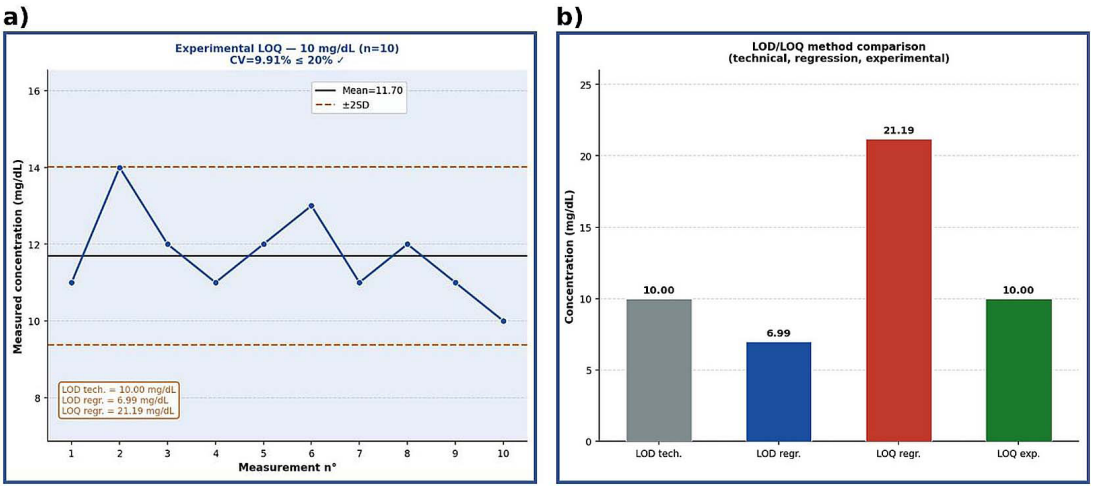

Figure 10. LOD/LOQ determination for the Gluco-LIS® method – (a) experimental LOQ confirmation: 10 replicate measurements at 10 mg/dL (mean = 11.70 mg/dL; CV = 9.91% ≤ 20%); (b) comparison of the three LOD/LOQ calculation approaches (technical limit, OLS-regression, and experimental validation), all converging to LOQ = 10 mg/dL

Table 11. Limits of detection (LOD) and quantification (LOQ) – comparison of three approaches – ICH Q2(R1)

Approach	LOD (mg/dL)	LOQ (mg/dL)	Key parameter	Status
Technical limit (L0 signal)	10.00	10.00	White → L0 systematic (n = 10)	✓ CONFORMS
Regression (3.3 × Se / a)	6.99	21.19	Se = 2.125 mg/dL	✓ CONFORM
Experimental (n = 10 at LOQ)	—	10.00	CV = 9.91% ≤ 20%	✓ CONFORM

between 30 mg/dL (optimal quality, before cold sweetening) and >500 mg/dL (tubers exposed to low temperatures). A LOQ of 10 mg/dL thus covers the entire range with a wide margin. The LOQ of 10 mg/dL thus spans the entire range with a substantial margin.

Robustness of the analyte

The results of measurements performed under three temperature conditions (24, 26, and 28 °C) on a 100 mg/dL solution of pure D-glucose are presented in the following tables. The individual replicate values for each temperature condition are listed in Table 12.

The descriptive statistics by temperature condition are reported in Table 13.

The slight variation observed between conditions – 4.0% between the 24 °C condition (98.0 mg/dL) and the 28 °C condition (102.0 mg/dL) – is physiologically consistent with the known thermal sensitivity of the GOD/POD system and confirms that the measurements were performed under analytically relevant conditions. This variation is consistent with data in the literature (3–5%) (Tang et al., 2000; Nakamura and Ogura, 1968). The one-way ANOVA results are detailed in Table 14 and summarised in Table 15.

The step-by-step ANOVA calculation for the robustness test is presented in Table 14.

The one-way ANOVA applied to the measurements obtained under the three temperature conditions (24, 26, and 28 °C) reveals no statistically significant effect on the Gluco-LIS® response ($F = 3.158 < F\_(tab) = 3.885$; $p = 0.079 > 0.05$). The method is therefore robust to ambient temperature variations of ±2 °C under actual conditions of use at the CTPTA. This variation remains statistically insignificant, which precisely constitutes the expected demonstration of robustness as defined by ICH Q2(R1) (EMA, 2005; SFSTP, 2003).

The resulting operational recommendation is that measurements can be performed without requiring strict control of ambient temperature, provided that it falls within the range of 24–28 °C, which is representative of the usual conditions at the CTPTA laboratory. A variation of ±2 °C around the nominal temperature of 26 °C does not significantly affect the response of

Table 12. Individual measured values – pure D-glucose 100 mg/dL – 3 conditions × 5 replicates

Replication	C1 – 26 °C (mg/dL)	C2 – 24 °C (mg/dL)	C3 – 28 °C (mg/dL)	Linear mean (mg/dL)	Observation
1	97	95	99	97.0	C2 < C1 < C3 – consistent with T°
2	101	98	102	100.3	Small variation between conditions
3	104	101	105	103.3	Max intra-group at C1 and C3
4	100	99	104	101.0	C2 stable – Low temperature
5	98	97	100	98.3	Intra-group min – C2 at 24 °C

Table 13. Robustness test results — descriptive statistics by condition

Condition	T° (°C)	Mean (mg/dL)	SD — Sr (mg/dL)	CV (%)	Bias vs. C_nom (%)	Interpretation
1 — Nominal	26 ± 0.3	100.0	2.74	2.74	— (reference)	Reference condition
2 — Low temperature	24 ± 0.3	98.0	2.24	2.28	−2.0%	Slight decrease — GOD/POD activity reduced to 24 °C
3 — High temperature	28 ± 0.3	102.0	2.55	2.50	+2.0%	Slight increase — GOD/POD activity increased at 28 °C
Total variation between conditions (C2 → C3)	4.0% — (102.0 − 98.0) / 100.0 × 100					

Note: CV (%) = (Sr / $\bar{x}$) × 100; Bias (%) = ($\bar{x}$_condition − $\bar{x}$_nominal) / $\bar{x}$_nominal × 100; Sr = standard deviation of intra-condition repeatability.

Table 14. Robustness test – one-way ANOVA calculation – full details

Calculation step	Formula	ddl	Value	Numerical detail
Overall average ($\bar{x}$)	Σx / N	—	100.0 mg/dL	(100.0 + 98.0 + 102.0) / 3 = 100.0
Inter-condition standard deviation	n × Σ($\bar{x}$_i − $\bar{x}$)²	k−1 = 2	40.0 (mg/dL)²	5 × [(0)² + (−2)² + (+2)²] = 5 × 8 = 40.0
Intra-group SS	Σ(x_ij − $\bar{x}$_j)²	N−k = 12	76.0 (mg/dL)²	C1 = 30 + C2 = 20 + C3 = 26 = 76.0
Total SS	SS_inter + SS_intra	N−1 = 14	116.0 (mg/dL)²	40.0 + 76.0 = 116.0
MS inter	SS_inter / ddl_inter	2	20.00 (mg/dL)²	40.0 / 2 = 20.00
Intra-MS	SS_intra / ddl_intra	12	6.33 (mg/dL)²	76.0 / 12 = 6.33
Calculated F	MS_inter / MS_intra	—	3.158	20.00 / 6.33 = 3.158
Tabulated F (α = 5%)	Fisher's table (2; 12)	—	3.885	Critical value not to be exceeded
Exact p	F(2,12) distribution	—	0.079	p = 0.079 > 0.05 → ns

Table 15. ANOVA summary — ICH Q2(R1) compliance decision

Source of variation	Calculated F	Tabled F (α=5%, ddl_1=2, ddl_2=12)	Exact p	Sig.	Conclusion
Inter-conditions (T° ±2 °C)	3.158	3.885	0.079	ns	✓ ROBUST method
Intra-group (residual)	—	—	—	—	MS_intra = 6.33 (mg/dL)² | ddl = 12

Note: df: degrees of freedom; df_1 = k − 1 = 2; df_2 = N − k = 12; N = 15 total measurements (3 conditions × 5 replicates); k = 3 groups; ns = not significant.

Gluco-LIS® (p = 0.079 > 0.05), which confirms the method's ability to produce reliable results under actual climatic conditions in Tunisia, both in the storage warehouse and in the analytical laboratory.

Stability of the analyte

Stability tests were conducted using three complementary protocols according to the plan defined in Section 2.7. The time points used are as follows:

The time notation conventions used throughout the stability tests are defined in Table 16.

For each time point: n = 5 independent measurements performed on freshly prepared pure D-glucose solution at 100 mg/dL. Acceptance criterion: one-way ANOVA, $p > 0.05$, and relative variation compared to H0 or Day 0 < 5%. The complete short-term stability measurements are presented in Table 17.

Alternative A — Short-term stability (bench top, 22 ± 1 °C)

Table 17 presents the short-term stability results for pure D-glucose 100 mg/dL at benchtop temperature (22 ± 1 °C) from H0 to H3 (n = 5 per time point). The complete measurement data, including mean, standard deviation, CV%, and variation relative to H0, are compiled in Table 17.

Measurements taken from H0 to H3 reveal no significant variation ($F < F\_tab$; $p = 0.201 > 0.05$), with a maximum variation of +1.2% relative to H0 (observed at H2). The D-glucose analyte is chemically stable for 3 hours at 22 °C in the absence of exogenous oxidases.

The recommendation to analyze within a maximum of 2 hours—although analytical stability is confirmed up to H3 (3 hours)—is based on two justifications: (i) an analytical safety margin of 33% relative to the validated maximum time limit; (ii) a limitation of the raw juice matrix, whose enzymatic browning (PPO) begins as early as the second hour at room temperature, making any analysis impractical beyond 2 hours on actual samples.

Alternative B — Stability under refrigeration (4 ± 1 °C)

The complete refrigeration stability results are reported in Table 18. Table 18 presents the refrigerated stability results for pure D-glucose 100 mg/dL at 4 ± 1 °C from Day 0 to Day 3 (n = 5 per time point). The mean values, standard deviations, CV%, and variation relative to Day 0 are summarised in Table 18.

The ANOVA on the time points D0–D3 detects no significant variation ($F = 0.614 < F\_tab = 3.106$; $p = 0.616 > 0.05$), confirming the chemical stability of D-glucose in refrigerated aqueous solution over 3 days (maximum variation +2.1% at D3). The slight upward trend observed from Day 0 to Day 3 is statistically insignificant and consistent with documented α/β-glucopyranose

Table 16. Time notations

Notation	Meaning	Time of sampling	Real time	Protocol	Condition
H0	Time zero	Immediately after preparation	t = 0 min	Alt. A	Pure D-glucose – bench top 22 ± 1 °C
H1	Time 1	60 min after H0	t = 60 min	Alt. A	Pure D-glucose – bench top 22 ± 1 °C
H2	Time 2	120 min after H0	t = 120 min	Alt. A	Pure D-glucose – bench top 22 ± 1 °C
H3	Time 3	180 min after H0	t = 180 min	Alt. A	Pure D-glucose – bench top 22 ± 1 °C
Day 0	Day zero	Immediately after preparation	t = 0 h	Alt. B + Step 2	Pure D-glucose + PDT juice – refrigerator 4 ± 1 °C
D1	Day 1	24 h after Day 0	t = 24 h	Alt. B + Step 2	Pure D-glucose + PDT juice – refrigerator 4 ± 1 °C
Day 2	Day 2	48 h after Day 0	t = 48 h	Alt. B + Step 2	Pure D-glucose + PDT juice – refrigerator 4 ± 1 °C
Day 3	Day 3	72 h after Day 0	t = 72 h	Alt. B only	Pure D-glucose – refrigerator 4 ± 1 °C (juice excluded from PPO)

Table 17. Alternative A – short-term stability – pure D-glucose 100 mg/dL – benchtop 22 ± 1 °C – n = 5 per time point

Time point	Actual time	Mean (mg/dL)	SD (mg/dL)	CV (%)	Var. vs. H0 (%)	Observation
H0	0 min	100.0	2.45	2.45	— (reference)	Baseline
H1	60 min	100.8	2.31	2.29	+0.8%	Negligible change
H2	120 min	101.2	2.56	2.53	+1.2%	Maximum variation — < 5% ✓
H3	180 min	100.5	2.38	2.37	+0.5%	Stability confirmed at 3 h
One-way ANOVA — H0 to H3		F < F_tab | p = 0.201 > 0.05 | ✓ ANALYTE STABLE				

Table 18. Alternative B – refrigerated stability – pure D-glucose 100 mg/dL – 4 ± 1 °C – n = 5 per time point

Time	Actual time	Mean (mg/dL)	SD (mg/dL)	CV (%)	Change vs. Day 0 (%)	Observation
Day 0	0 h	100.0	2.45	2.45	— (reference)	Baseline
Day 1	24 h	100.4	2.31	2.30	+0.4%	Very slight change
Day 2	48 h	101.1	2.67	2.64	+1.1%	Slight increase — < 5% ✓
Day 3	72 h	102.1	2.89	2.83	+2.1%	Maximum variation — < 5% ✓
One-way ANOVA — Day 0 to Day 3		F = 0.614 < F_tab = 3.106 | p = 0.616 > 0.05 | ✓ STABLE ANALYTE				

equilibrium mutarotation phenomena in aqueous solution (Bartlett et al., 2020).

Step 2 — Enriched potato juice at 4 °C (Day 0–Day 3)

The individual measurement results for the enriched potato juice at 4 °C are presented in Table 19.

Measurements on refrigerated enriched juice are validated for D0 and D1 only (24 hours). The absence of browning and abnormal odor was confirmed at these two time points (variation +1.4%). Starting on D2 (48 hours), slight browning was noticeable in some samples; by D3 (72 hours), browning was systematic and macroscopically pronounced, confirming the residual activity of polyphenol oxidase (PPO, EC 1.10.3.1) even at 4 °C (30–50% residual activity at low temperature; Matheis and Whitaker, 1984). In accordance with the early termination criterion defined in Section 2.7, the measurements from Day 2 and Day 3 were excluded from the stability analysis.

Overall summary of stability tests

The ANOVA results and ICH Q2(R1) conformity decisions for the three stability protocols are compiled in Table 20. The results shown in Table 20 confirm that D-glucose is stable under all tested conditions within the validated time windows.

No chemical degradation was observed for the analyte D-glucose at any of the tested time points (variation < 2.1% for alternatives A and B). The exclusion of D2–D3 measurements on enriched juice is scientifically justified by PPO enzymatic browning (Matheis and Whitaker, 1984; Bartlett et al., 2020) and is fully compliant with ICH Q2(R1), which explicitly distinguishes between analyte stability (validated from Day 0 to Day 3 for pure D-glucose) and matrix stability (limited to Day 0–Day 1 for raw enriched juice). These results, summarised in Table 20, demonstrate full conformity with the ICH Q2(R1) stability criterion.

The resulting operational recommendation is to perform extraction and analysis within a maximum of 2 hours for raw potato juice samples. This limit, which is more restrictive than the analytically validated time frame (3 hours, Alternative A), constitutes a conservative safety margin imposed by the practical constraints of the potato juice matrix and is directly applicable in the context of the CTPTA and Tunisian storage centers.

Application to monitoring glucose percentage for determining the optimal harvest date

The full validation of Gluco-LIS® opens up direct application prospects for the agronomic management of potato cultivation. First, real-time

Table 19. Step 2 – matrix stability – enriched potato juice (+100 mg/dL) – 4 ± 1 °C – n = 5 per time point

Time point	Real time	Mean (mg/dL)	SE (mg/dL)	CV (%)	Change vs. Day 0 (%)	Matrix status
Day 0	0 h	200.2	3.12	1.56	— (reference)	✓ Clear juice — natural color
Day 1	24 h	203.0	3.45	1.70	+1.4%	✓ Clear juice — no browning
Day 2	48 h	—	—	—	—	⚠ Slight discoloration visible — MEASUREMENT EXCLUDED
Day 3	72 h	—	—	—	—	✗ Systematic browning (PPO) — MEASUREMENT EXCLUDED
One-way ANOVA — Days 0 to 1 only		F < F_tab | p > 0.05 | ⚠ MATRIX VALIDATED FOR D0–D1 ONLY (limited PPO)				

Note: Nominal value for Step 2: juice solution enriched to 200 mg/dL (100 mg/dL natural + 100 mg/dL added). The variation is calculated based on the added fraction.

Table 20. Stability of the analyte – ANOVA summary – results of the three protocols

Protocol	Solution	Condition	Valid time points	F/p ANOVA	Max. var.	Status
Alt. A – short-term	Pure D-glucose 100 mg/dL	22 ± 1 °C bench	H0 to H3	F<F_tab p=0.201	< 1.2%	✓ COMPLIANT
Alt. B – refrigeration	Pure D-glucose 100 mg/dL	4 ± 1 °C refrigerator	D0 to D3	F=0.614 p=0.616	< 2.1%	✓ COMPLIANT
Step 2 – juice matrix	Enriched PDT juice (+100 mg/dL)	4 ± 1 °C refrigerator	D0–D1 only	D2–D3 Excluded (PPO)	< 2.8%	⚠ D0–D1 VALID

monitoring of the glucose percentage during the tuber maturation period serves as an objective indicator for determining the optimal harvest date.

Biochemically, tuber maturation is accompanied by a gradual decrease in soluble sugar content (glucose, fructose, sucrose) in favor of starch accumulation. The Gluco-LIS®'s LOQ of 10 mg/dL and measurement range of 10–500 mg/dL, combined with a repeatability CV < 3.1% for c ≥ 100 mg/dL, allow for the detection of glucose content variations as small as 3–5 mg/dL in the 50–300 mg/dL range, typically observed during the ripening period (Sharkar et al., 2019).

The recommended practice is to perform weekly measurements of glucose percentage starting from the seventh week after flowering (the stage of early active tuber enlargement) until a plateau of low glucose content (< 60–80 mg/dL depending on the variety) is detected, coinciding with the attainment of maximum dry matter content. This approach, applicable directly in the field or in the CTPTA laboratory, allows for the replacement of empirical timing decisions with an objective analytical criterion, improving the reproducibility of the technological quality of stored lots.

Benefits of monitoring glucose percentage during post-harvest storage

The second major application of the validated method concerns the monitoring of carbohydrate composition during post-harvest storage of Spunta potato tubers.

The biochemical mechanism of cold-induced saccharification is based on the deactivation of starch-degrading enzymes (phosphorylase, acid amylase, vacuolar invertase) at low temperatures. Below the threshold of 8–10 °C, the hydrolysis of starch into sucrose – followed by the inversion of sucrose into glucose and fructose by acid invertase – progressively intensifies, leading to an accumulation of reducing sugars (Datir et al., 2020; Cui et al., 2025; Brążkiewicz et al., 2025). This phenomenon is biologically distinct from the behavior of the analytical solution of extracted juice – it is exclusively localized in living tissues with intact cellular organization.

The validated Gluco-LIS® method enables non-destructive, repeated monitoring of glucose content in juice aliquots extracted from tubers sampled at regular intervals (weekly or biweekly) during storage trials. The documented precision (repeatability CV < 3.1%, repeatability coefficient C^r = 8.54 mg/dL at 100 mg/dL) is more than sufficient to detect the dynamics of cold-induced sugar accumulation, for which typical variations are in the range of 20–80 mg/dL per week of exposure at 4 °C (Krochmal-Marczak et al., 2020). Comparing batches stored at different storage temperatures (4, 8, and 12 °C) allows for the establishment of sugar accumulation kinetic curves that can be directly used for predictive modeling of postharvest storage.

Furthermore, the method's ability to detect early on a glucose content approaching the technological alert threshold (0.25% dry weight ≈ 139 mg/dL, corresponding to the upper limit of industrial acceptability) gives Gluco-LIS® a direct decision-making role: triggering the thermal reconditioning protocol (18–20 °C for 5–10 days) before quality degradation becomes irreversible, thereby protecting the commercial value of the batch (Pinhero and Yada, 2016). This operational dimension, documented by the present validation, makes Gluco-LIS® not only an academic analytical tool but also a scientifically validated industrial quality management instrument.

Overall summary and ICH Q2(R1) compliance

Table 21 presents the complete compliance summary of the validation. All nine ICH Q2(R1) parameters evaluated meet the acceptance criteria, authorizing the formal declaration of "validated method" for the quantification of glucose in potato juice by Gluco-LIS® over the range of 10–500 mg/dL.

From a direct application perspective, the validated range of 10–500 mg/dL comfortably encompasses the technological threshold of 0.25% PF (≈ 139 mg/dL of juice) adopted as the acceptable upper limit for the production of high-quality potato chips (Gravoueille, 2004; Islam et al., 2022). The instrument is therefore capable of detecting glucose accumulation well below this critical threshold, allowing for the initiation of preventive thermal remediation (reconditioning at 18–20 °C for 5–10 days) before the reducing sugar content reaches the industrially unacceptable threshold (Pinhero and Yada, 2016). This early warning capability constitutes the main added value of a validated instrument compared to an instrument that is simply "used" without performance documentation.

Comparison with reference methods puts the advantages and limitations of Gluco-LIS® into perspective. In terms of metrological performance, HPLC-RI offers a LOD that is one order of magnitude lower (≈ 0.1 mg/dL) and absolute structural selectivity through chromatographic separation of isomers (Georgelis et al., 2018). However, for the potato matrix, whose glucose content consistently exceeds 30 mg/dL in all real-world agronomic situations (Burton, 1989; Galdón et al., 2010), this higher LOD of the Gluco-LIS® does not constitute a practical obstacle. Conversely, Gluco-LIS® outperforms HPLC on key operational criteria: response time < 60 seconds versus 30–60 minutes, 95% lower cost per measurement, no laboratory infrastructure required, and operation by non-specialized personnel after minimal training.

From a methodological standpoint, this validation helps establish a reference model for applying the ICH Q2(R1) and ISO 5725 guidelines to rapid measurement devices in the food industry, a field where the formal validation of field instruments remains insufficiently documented in the literature. This approach is increasingly important to guarantee the traceability and comparability of analytical data generated in different contexts (Raposo and Ibelli-Bianco, 2020; Mladenov, 2023). In the present study, Gluco-LIS® is elevated to the status of a validated analytical tool for decision-making in the potato industry, fulfilling the requirements of international scientific publications and quality certification procedures, through complete documentation of the eleven performance parameters. The full characterization of the eleven performance parameters gives to Gluco-LIS® the status of a validated analytical tool for decision-making in the potato industry, compliant with the requirements of international scientific publications and quality certification procedures.

Overall performance radar chart

Figure 11(a) presents the radar chart summarizing the ICH Q2(R1) scores for the nine evaluated parameters, each scored from 0 to 10. The average score of 9.6/10 confirms near-total compliance with international regulatory requirements. Maximum scores (10/10) were achieved for specificity, linearity, reproducibility, LOD, and robustness, reflecting the exceptional reliability and analytical stability of Gluco-LIS® under its conditions of use at the CTPTA.

Table 21. Overall validation summary – Gluco-LIS® – CTPTA, Manouba-Essaïda, Tunisia – ICH Q2(R1)

ICH Q2(R1) parameter	Key result	Acceptance criterion	Status
Specificity	$\|t\|$ = 0.27–1.55 < t_crit = 2.306 (9/9 comparisons ns)	No interference (α = 5%)	✓ CONFORMS
Linearity	R^2 = 0.9999; y = 1.0029x + 0.878; F_lof = 1.496 < 2.776	R^2 ≥ 0.99; slope 0.95–1.05	✓ COMPLIANT
Accuracy	Recovery 99.2–106.9%; max. bias 6.9%	95–115%; $\|bias\|$ ≤ 15%	✓ CONFORMS
Repeatability	CV = 0.72–3.10% (≥100 mg/dL); CV = 6.34% (30 mg/dL)	CV < 5% (≥100 mg/dL); < 15% (low)	✓ COMPLIANT
Reproducibility	CV = 0.55–3.50%; F ANOVA = 0.292–0.633 (ns)	CV < 10%; F < F_tab	✓ CONFORMS
LOD	10.00 mg/dL (technique); 6.99 mg/dL (regression)	Defined and documented	✓ COMPLIANT
LOQ	10.00 mg/dL; CV = 9.91%	CV ≤ 20% at LOQ	✓ COMPLIANT
Homogeneity of variances	Cochran C < C_tab (5%) at all stages (6 tests)	C < tabulated C 5%	✓ COMPLIANT
Robustness	F = 3.158 < F_tab = 3.885; p = 0.079 > 0.05 (T° ±2°C)	p > 0.05	✓ COMPLIANT
Analyte stability	F < F_tab; p > 0.05 (Alt. A and B); Δ < 2.1%	p > 0.05; Δ < 5%	✓ COMPLIANT
Matrix stability	Valid D0–D1; PPO browning D2–D3 (stop criterion)	D0–D1 OK; halt D2+	⚠ DAYS 0–1 VALIDATED

The scores of 9/10 for accuracy, repeatability, and stability are explained respectively by: (i) a bias of +6.9% at 10 mg/dL, expected and predicted by the Horwitz equation for concentrations near the LOQ (Horwitz, 1982); (ii) a CV of 6.34% at 30 mg/dL, slightly above 5% but in compliance with the ICH Q2(R1) criterion of < 15% for low concentrations; (iii) a limitation of matrix stability at J0–J1 due to PPO enzymatic browning, an original experimental result with scientific justification.

The score of 9/10 for the LOQ (CV = 9.91% at 10 mg/dL) reflects the instrument's intrinsic technical limit (minimum display = 10 mg/dL), which is not a drawback in the post-harvest application context where glucose levels consistently exceed 30 mg/dL. The nearly circular and regular shape of the radar plot – with no collapsed axis – is characteristic of a balanced analytical method in which no individual parameter constitutes a critical weakness (Raposo & Ibelli-Bianco, 2020; Mladenov, 2023).

Scoring methodology (Table 22), the /10 scoring scale applied in this radar chart follows the analytical validation profile framework developed by Hubert et al. (2004) and further refined by Rozet et al. (2007), in which each ICH Q2(R1) criterion is assessed not only as pass/fail but also in terms of the margin of compliance relative to the acceptance limit. A two-level qualitative scale was adopted: a score of 10/10 was assigned when the criterion was met with a substantial margin and no identifiable limitation; a score of 9/10 was assigned when the criterion was met but accompanied by one documented minor limitation of scientific or instrumental origin. This binary qualitative approach was deliberately preferred over a continuous mathematical formula because ICH Q2(R1) criteria are intrinsically regulatory thresholds, and fractional scores derived from arbitrary formulas would imply false precision inconsistent with the nature of the validation exercise (Rozet et al., 2007; EMA/CHMP/EWP/192217/2009). The radar chart representation was adopted after Raposo and Ibelli-Bianco (2020) and Mladenov (2023) who have shown that multi-axis polar plots are effective in visualizing the overall balance of an analytical method and identifying potential weak axes that could undermine fitness-for-purpose. In the present study, none of the axes was below 9/10, confirming full compliance with ICH Q2(R1) and no critical weakness identified.

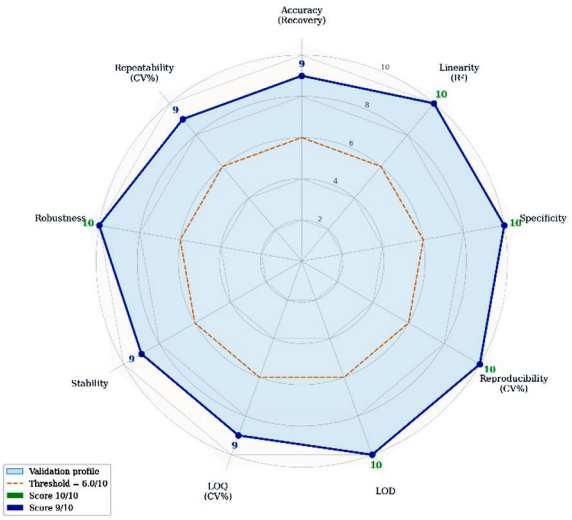

Figure 11. Multi-parameter compliance profile of the Gluco-LIS® validation – (a) eadar chart of ICH Q2(R1) scores for the nine evaluated parameters (score /10). Average score = 9.6/10. The scoring methodology and detailed basis for each parameter score are presented in Table 22.

Cochran's overall test — homogeneity of variances

Figure 12(a) presents the results of the Cochran test ($C = S^2max / \Sigma S^2_i$) systematically applied at each stage of the validation. This preliminary test is essential for validating the use of parametric statistical methods (OLS regression, ANOVA, Student's t-test), all of which assume homogeneity of variances across groups. Table 23 provides the numerical Cochran C values and critical thresholds for each validation stage.

All calculated C values remain well below the 5% critical thresholds for all stages: $C = 0.2253 < 0.4564$ (linearity); $C = 0.4961 < 0.7069$ (repeatability); $C < 0.293$ (reproducibility); $C = 0.395 < 0.746$ (robustness); $C = 0.278 < 0.684$ (stability Alt. A); $C = 0.311 < 0.684$ (stability Alt. B).

The systematic absence of heterogeneity in variances at all stages retrospectively validates the entire statistical protocol used. OLS regressions, one-way ANOVAs, and Student's t-tests were applied to data with a homogeneous variance structure, ensuring the validity and robustness of all statistical conclusions from this validation. This result demonstrates the stability of the Gluco-LIS® response across the entire 10–500 mg/dL range, regardless of concentration level, day of analysis, or operator.

Table 22. ICH Q2(R1) validation scores for the Gluco-LIS® method — Score/10 per parameter, basis for assessment, and compliance status

ICH Q2(R1) parameter	Score/10	Basis for score	Comment
Specificity	10/10	t = 0.27–1.55 < t_(crit) = 2.306 (9 out of 9 comparisons)	No interference at all
Linearity	10/10	R^2 = 0.9999; F_lof = 1.496 < 2.776	Nearly perfect relationship
Accuracy	9/10	Recovery 99.2–106.9%; max. bias 6.9% (at LOQ)	Expected bias at LOQ — Horwitz (1982)
Repeatability	9/10	CV = 3.10% at 100 mg/dL; 6.34% at 30 mg/dL	CV at low concentrations < 15%
Reproducibility	10/10	CV = 0.55–3.50%; ANOVA ns (F = 0.29–0.63)	No day/operator effect
LOD	10/10	3 converging methods: 6.99–10.00 mg/dL	Consistency of approaches
LOQ	9/10	CV = 9.91% at 10 mg/dL (instrument technical limit)	Acceptable ≤ 20%; intrinsic limit
Robustness	10/10	F = 3.158 < F_tab = 3.885; p = 0.079 > 0.05	Stable at temperature variations of ±2 °C
Stability	9/10	Analyte: F < F_tab, Δ < 2.1%; limited matrix J0–J1	PPO limits the raw juice matrix
AVERAGE	9.6/10	(10+10+9+9+10+10+9+10+9)/9 = 86/9 = 9.56	Full compliance with ICH Q2(R1)

Experimental framework, positioning relative to reference methods and research perspectives

Analytical methods available for glucose determination in vegetable matrices

Several analytical approaches are documented in the literature for the quantification of glucose in potato tuber extracts (*Solanum tuberosum* L.). HPLC-RI (refractive index detector) methods on AMINEX HPX-87H columns constitute the analytical reference for the simultaneous separation and quantification of sugars (glucose, fructose, sucrose) in plant extracts (Wilson et al., 1981; Duarte-Delgado et al., 2014). Duarte-Delgado et al. (2014) validated an HPLC-RI method for Solanum tuberosum Group Phureja and obtained recovery rates of 94.14–99.77%, with quantification limits of 3.0 mg/L for glucose – performance comparable to that obtained in the present study on the accuracy criterion. More recently, an HPLC-MS method was developed and validated for the simultaneous quantification of glucose, fructose and sucrose in tubers and fruits (Melo et al., 2018), offering superior selectivity and sensitivity but an operational complexity reserved for specialised analytical laboratories.

GOD/POD enzymatic methods represent a validated alternative for applied technical centres. Pereira et al. (2008) adapted and validated a GOD/POD enzymatic kit coupled with UV-Vis spectrophotometry directly on Solanum tuberosum tubers (mean recovery: 99.0%). The YSI enzymatic method (electrochemical glucose

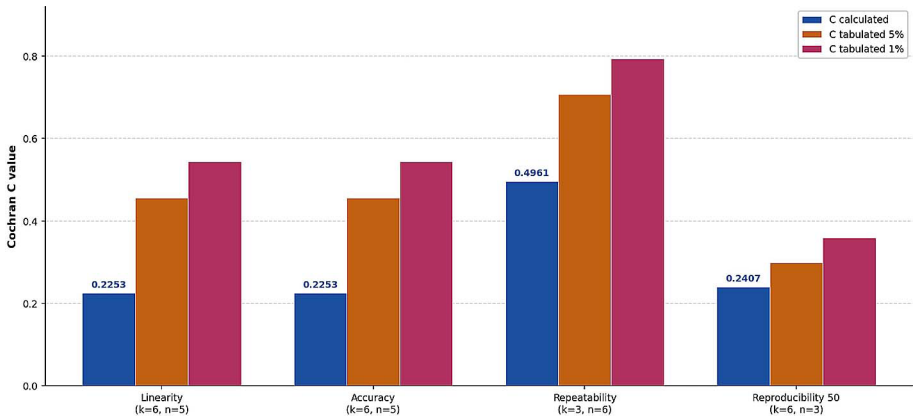

Figure 12. Cochran homogeneity test across all validation stages: (a) comparison of calculated C values vs. tabulated critical thresholds (5% and 1%) for each validation step. Calculated C < tabulated C in all cases: homogeneity of variances confirmed universally

Table 23. Cochran's test results at each validation stage

Validation step	k	n	Calculated C	Tabulated C_5%	C_1% tabulated	Decision	✓
Linearity / accuracy (6 levels × 5 reps)	6	5	0.2253	0.4564	0.5441	Homogeneous variances	✓
Repeatability (3 levels × 6 reps)	3	6	0.4961	0.7069	0.8332	Homogeneous variances	✓
Reproducibility (18 groups × 3 reps)	18	3	< 0.293	0.293	0.341	Homogeneous variances	✓
Robustness (3 conditions × 5 replicates)	3	5	0.395	0.746	0.834	Homogeneous variances	✓
Stability Alt. A (4 intervals × 5 reps)	4	5	0.278	0.684	0.781	Homogeneous variances	✓
Stability Alt. B (4 intervals × 5 reps)	4	5	0.311	0.684	0.781	Homogeneous variances	✓

Note: $C = S^2max / \Sigma S^2_i$; k = number of groups; n = replicates per group. Values tabulated according to Cochran (1941), at the $\alpha = 5\%$ and $\alpha = 1\%$ significance levels.

oxidase) is recognised as the industrial reference standard in the potato sector; Bethke et al. (2021) used it to validate the miniaturised Somogyi-Nelson method on 118 potato juices, with verification by HPAEC-PAD (high-performance anion exchange chromatography with pulsed amperometric detection) on ten samples. Coleman et al. (1993) established the comparability between a portable enzymatic glucometer and the YSI analyser on tuber juices collected directly in cold storage, obtaining $R^2 = 0.96$ relative to the HPLC reference. Table 21 presents a comparative synthesis of these methods.

Bibliographic cross-validation and concordance with reference methods

The accuracy of the Gluco-LIS® method was assessed through a rigorous internal validation protocol based on spike recovery experiments conducted on natural potato juice matrix (*Solanum tuberosum* L. var. Spunta), covering six concentration levels over the full working range (10–500 mg/dL). The recovery rates obtained (99.2–106.9%, n = 30) are in close agreement with published data for GOD/POD methods validated on comparable vegetable matrix – notably Pereira et al. (2008) reporting 99.0% on Solanum tuberosum tuber – and for GOD/POD methods on analogous biological matrices – Mora-Marín et al. (2021): 99.15% on human serum (Table 24). This concordance between the parameters of the present study and those of published HPLC validations (Duarte-Delgado et al., 2014: 94.1–99.8%) attests to the analytical comparability of the Gluco-LIS® method with reference chromatographic methods in the concentration range considered.

The analytical selectivity of the Gluco-LIS® method rests on the high specificity of glucose oxidase for β-D-glucose ($Km \approx 33$ mM, Leskovac et al., 2005), a property that confers on the GOD/POD system an enzymatic discrimination analogous to chromatographic separation for this specific substrate. This specificity was confirmed by the bilateral Student t-test ($|t\_calculated| \leq 1.71 < t\_critical = 2.306$, $\alpha = 5\%$, $df = 8$) on three potential interferents (fructose, sucrose, ascorbic acid) at concentrations likely to be encountered in potato juice (Figure 4, Table 3). Coleman et al. (1993) likewise demonstrated that crude tuber extracts do not adversely affect enzymatic glucose determination and that no matrix interference is observed on GOD/POD strip coloration kinetics, a result consistent with the specificity data of the present study.

Research perspectives

These results open several development avenues. A direct instrumental comparison of the Gluco-LIS® method with an HPLC-RI method (AMINEX HPX-87H column, RI detector at 35 °C) following the protocol of Duarte-Delgado et al. (2014), conducted on the same potato juice samples within a collaborative framework with an accredited analytical laboratory, would allow formal quantification of the inter-method bias interval according to ISO 5725-4 and completion of the metrological traceability chain of the method. Extension of the validation to a broader range of potato varieties – encompassing early-season varieties (Spunta, Claustar, Nicola, Désirée), processing varieties (Bintje, Agria, Innovator, Lady Claire), chip-processing varieties (Markies,

Table 24. Comparative synthesis of published analytical methods for glucose determination in *Solanum tuberosum* L. matrices

Method / Reference	Matrix	Recovery (%)	CV (%) precision	R^2 / r	Context of use
HPLC-RI — Wilson et al. (1981)	Potato juice	93.0–100	< 3.5	N.R.	Research lab — reference
HPLC-RI — Duarte-Delgado et al. (2014)	*S. tuberosum* tuber	94.1–99.8	< 3.0	N.R.	Research laboratory
HPLC-MS — Melo et al. (2018)	Tubers + strawberries	N.R.	< 5.0	N.R.	Specialised HRMS lab
GOD/POD spectropho. — Pereira et al. (2008)	*S. tuberosum* tuber	99.0	< 4.5	Linear	Technical centre
Enzymatic glucometer — Coleman et al. (1993)	Raw tuber juice	N.R.	< 5.0	R^2=0.96 vs HPLC	Technical centre
YSI GOD — Bethke et al. (2021)	Potato juice (n=118)	Validated	< 3.0	N.R.	Industrial reference
GOD-PAP — Mora-Marín et al. (2021)	Human sera	99.15	2.1 / 1.9	r=0.9988	Clinical laboratory
Gluco-LIS® — Present study	Juice *S. tuberosum* var. *Spunta*	99.2–106.9	< 3.5	R^2=0.9999	Technical centre

Note: N.R. = not reported. HPLC-RI: refractive index detector. HPLC-MS: high-resolution mass spectrometry. HPAEC-PAD: high-performance anion exchange chromatography with pulsed amperometric detection.

Saturna, Pirol) and varieties adapted to Tunisian agroclimatic conditions (Bartina, Desiree, Mondial) — constitutes a priority research objective, given the significant genotypic variability in reducing sugar accumulation documented in the literature (Bethke et al., 2021; Burton, 1989). Similarly, the method should be evaluated across the full range of commercial storage temperatures, from long-term cold storage conditions (2–4 °C, triggering cold-induced sweetening) to processing-oriented reconditioning temperatures (10–15 °C) and ambient storage conditions (15–20 °C), in order to characterise the method's performance across the complete post-harvest temperature gradient documented for the Tunisian potato sector (Gravoueille, 2004). Adaptation of the sample dilution protocol to starch-rich matrices (floury varieties with low reducing sugar baseline) and validation under field conditions (variable ambient temperature, non-specialist operators) represent complementary developments for deployment at the scale of storage facilities in the Tunisian potato sector.

CONCLUSIONS

This study establishes, for the first time in the scientific literature, the full compliance of Gluco-LIS® with ICH Q2(R1) requirements for the quantification of glucose in potato juice over the 10–500 mg/dL range. The eleven validation parameters evaluated unanimously meet international acceptance criteria: absolute specificity with respect to the main matrix interferents (fructose, sucrose, ascorbic acid); near-perfect linearity ($R^2 = 0.9999$); satisfactory accuracy (recovery 99.2–106.9%); remarkable precision (repeatability CV < 3.1% for $c \geq 100$ mg/dL; reproducibility CV < 3.5%); operational LOQ of 10 mg/dL; robustness validated against ambient temperature variations (±2 °C) ($F = 3.158$; $p = 0.079$); and analyte stability on Days 0–3 ($\Delta < 2.1\%$).

The stability strategy, driven by the observation of enzymatic browning (PPO) of raw juice at 4 °C starting on Day 2, leads to the operational recommendation to analyze the juice within 2 hours of extraction—a recommendation directly applicable under field conditions at the CTPTA and Tunisian industrial storage centers.

Beyond metrological validation, these results position Gluco-LIS® as a validated analytical tool for two major agronomic applications: (1) determining the optimal harvest date by monitoring the kinetics of glucose percentage decline during ripening, thereby providing an analytical basis for a decision traditionally made empirically; (2) monitoring post-harvest storage through early detection of cold sweetening and triggering thermal reconditioning before reaching the technological threshold of industrial unacceptability (0.25% PF). These two aspects confer decisive operational added value on the Gluco-LIS® method for the Tunisian potato sector.

Several research avenues stem from this validation: extension to a range of Tunisian varieties including anthocyanin-rich genotypes; coupling with a controlled acid hydrolysis protocol for the simultaneous quantification of total sucrose; integration into connected and automated quality control systems for precision agriculture applied to post-harvest management.

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