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Evaluation of Enzyme Activity Measurement Methods: Application of a Glucometer as a Rapid and Low-Cost Alternative to HPLC

Saloomeh Shoaei Naeeni[1], Bijan Bambai[2,*], Bagher Yakhchali[3], Parvaneh Saffarian[1], Zarrin Minuchehr[2]

[1] *Department of Biology, SR.C., Islamic Azad University, Tehran, Iran*
[2] *Systems Biotechnology Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Shahrak-e Pajoohesh, Km 15, Tehran - Karaj Highway, Tehran, Iran*
[3] *Energy and Environmental Biotechnology Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Shahrak-e Pajoohesh, Km 15, Tehran - Karaj Highway, Tehran, Iran*

***Corresponding authors:** bambai@nigeb.ac.ir

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Abstract

Glucose isomerase (GI) catalyzes the reversible conversion of glucose to fructose and plays a major role in producing high-fructose corn syrup (HFCS). Accurate and rapid measurement of GI activity is essential for process optimization and quality control. This study compared two analytical methods—high-performance liquid chromatography (HPLC) and a portable glucometer—for determining GI activity in recombinant *Escherichia coli* BL21 cultures grown under various conditions. Enzyme activity was quantified by monitoring glucose consumption and fructose production. Statistical analysis, including regression and independent *t*-tests, revealed a strong correlation between glucometer and HPLC results ($R^2 = 0.96$, $p = 0.0001$) with no significant difference in mean activities. The findings demonstrate that the glucometer, despite its simplicity and low cost, provides reliable data comparable to HPLC. This approach provides a rapid and cost-effective alternative for routine glucose isomerase activity screening in laboratory and industrial settings.

Keywords: Enzymatic assay; Fructose; Glucose isomerase; Glucometer; HPLC; Quantification

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1. Introduction

Glucose isomerase (GI) (EC 5.3.1.5), also known as xylose isomerase, is a key enzyme in carbohydrate metabolism that catalyzes the reversible interconversion of glucose and fructose. Glucose isomerase plays a central role in the industrial production of high-fructose corn syrup (HFCS), a widely used sweetener in beverages, baked goods, canned fruits, and dairy products [1]. Owing to its high sweetness, solubility, and stability, HFCS is a preferred alternative to sucrose in many food formulations [2]. Beyond food applications, GI is employed in bioethanol production, where it facilitates the conversion of glucose and xylose into fermentable sugars for renewable fuel generation [3]. Additionally, it contributes to the synthesis of rare sugars, such as D-tagatose and D-xylose, which have potential applications in pharmaceuticals and functional foods [4]. These diverse applications highlight the industrial importance of this enzyme and its role in advancing sustainable bioprocesses. In countries like Iran, where industrial enzyme production is still developing, GI provides strategic value by reducing reliance on imports and enabling the domestic manufacturing of sweeteners and bio-based products. Its integration into local fermentation platforms can support food security, expand biotechnological capabilities and stimulate innovation in enzyme engineering. Therefore, reliable and scalable measurement of GI activity are crucial for both research and industrial applications, as they enable the optimization of fermentation processes, evaluation of enzyme kinetics, and monitoring of quality control in bioprocesses [5].

Various analytical methods have been developed to measure GI activity, including spectrophotometric, chromatographic, and biochemical techniques [5]. In the spectrophotometric approach, enzyme activity is determined by monitoring changes in absorbance at a wavelength of 405 nm, using chromogenic substrates such as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+). This method is simple, reliable, and well-suited for food sample analysis [5]. Spectrophotometry enables effective monitoring of glucose conversion to fructose by measuring the change in optical absorbance at a specific wavelength during the progression of the reaction. By analyzing these absorbance changes over time, researchers can quantify and calculate the specific enzyme activity. Chromatography is one of the most precise and widely used methods for assessing glucose isomerase activity, enabling the investigation of enzyme kinetics and inhibition through the separation and quantification of glucose and fructose produced during the reaction. Techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and ion-exchange chromatography allow for the accurate and quantitative measurement of glucose and fructose in samples.

In HPLC, the separation of glucose and fructose is achieved based on their retention times within the chromatographic column. The selection of an appropriate column and optimization of the stationary and mobile phase conditions are critical for achieving high-resolution separation. When coupled with detectors such as refractive index detectors (RID), pulsed amperometric detectors (PAD), and evaporative light scattering detectors (ELSD), this method offers high sensitivity and precision, facilitating detailed kinetic studies and inhibitor profiling [6–8].

In gas chromatography, volatile and non-volatile sugars are analyzed after derivatization to volatile compounds. In contrast, ion-exchange chromatography separates glucose and fructose based on differences in their electrical charges. Mass spectrometry (MS) can be employed following initial chromatographic separation to enhance identification accuracy. Collectively, these chromatographic techniques, through the analysis of chromatogram peaks, enable the precise quantification of glucose-to-fructose conversion by glucose isomerase and serve as powerful tools for studying enzymatic performance under various conditions.

Biochemical methods are key tools for assessing glucose isomerase activity, often performed using commercial kits based on specific enzymatic reactions. These methods enable the precise measurement of enzyme activity through the generation of colored or fluorescent products that can be quantified using instruments such as spectrophotometers and fluorometers. Commonly used enzymes in this context include glucose oxidase (GOx) and glucose dehydrogenase (GDH). These enzymes catalyze the conversion of glucose to gluconic acid, producing hydrogen peroxide or NADPH, which leads to a color change or fluorescence. The signal intensity is directly proportional to the glucose concentration [9,10]. Recent adaptations have extended glucometers to enzymatic kinetics [e.g., invertase/sucrose hydrolysis in educational labs] and point-of-care diagnostics beyond glucose, though direct validation against HPLC for isomerase activity remains limited.

The quick furfural test is a simple and rapid method for detecting fructose. In this assay, the addition of ethanol 1-naphthol and hydrochloric acid to a diluted sugar solution, followed by boiling, resulted in the formation of a purple color within 30 s, confirming the presence of fructose. In the field of blood glucose monitoring, the glucose oxidase enzymatic method is widely applied. Blood glucose test strips impregnated with glucose oxidase selectively detect glucose and produce a measurable color change via an enzymatic reaction. These strips can distinguish glucose from other structurally similar sugars, such as maltose, lactose, and glycogen, offering high accuracy in glucose quantification. Additionally, the use of carbon nanodots as stable fluorophores under various conditions has enhanced the sensitivity and precision of fluorescence-based methods [11]. Glucometers, originally designed for blood glucose monitoring, have recently gained attention for their applications in the food industry. These devices typically utilize enzymes such as glucose oxidase (GOx), glucose dehydrogenase (GDH), or pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH) and measure glucose concentration within less than a minute using amperometric or colorimetric methods [5,12]. The glucose levels measured by glucometers and laboratory chemical methods often differ in accuracy [5], primarily because of the use of different blood sample types. Home glucometers analyze whole blood (including cells and plasma) collected from capillaries, whereas laboratory methods determine glucose concentration using plasma or serum from venous blood samples. In laboratory settings, red blood cells and other components are separated from plasma, and glucose is measured exclusively in the plasma fraction. Consequently, glucometer readings from whole blood tend to be lower than laboratory plasma glucose measurements. Most glucometers report results with a typical deviation of ±10% compared with laboratory values.

Several factors influence glucometer accuracy, including device calibration, ambient temperature, blood sample volume applied to the test strip, presence of specific medications, hematocrit levels, device cleanliness, and the expiration of the test strip. Therefore, it is recommended to compare glucometer readings with laboratory results once or twice a year, when possible.

Another issue with glucometer-based glucose measurements is the variability in readings from the same blood sample across different measurements. Even when using the same device, the results may differ between samples. Moreover, results from two different glucometers cannot be directly compared unless both devices are calibrated identically. Despite these limitations, glucometer readings provide approximate glucose levels.

Common sources of error in glucometer readings include insufficient blood volume to fully fill the test strip channel, strip sensitivity to moisture and high temperatures, expired test strips, mismatched strip and device codes, dirty sensors, physical damage to the device, and exposure to extreme conditions [13–15]. Environmental factors, such as altitude, temperature, and humidity, can also unpredictably affect glucose readings and measurement accuracy.

The measurement of enzymes, including glucose isomerase, is of great importance in biological and medical research, as well as in the food and pharmaceutical industries. Various methods for measuring glucose isomerase activity ensure accurate and reliable results in research and industrial applications. Selecting an appropriate method based on the experimental needs and laboratory conditions is crucial. Given the advantages and limitations of each technique, careful selection of the appropriate tool is essential for obtaining precise results.

In this study, glucose isomerase activity was assessed and compared using two instruments: HPLC and a glucometer. The primary objective was to evaluate the accuracy and reliability of the glucometer in comparison to the reference method and to assess its potential as a rapid, cost-effective, and reliable tool for measuring glucose isomerase activity under laboratory and industrial conditions. Ultimately, the performance of the glucometer relative to HPLC was evaluated through a statistical analysis of the data.

HPLC was selected as the reference method due to its status as the gold standard for accurate separation and quantification of glucose and fructose in complex matrices, allowing direct measurement of both substrate consumption and product formation [6–8]. In contrast, the portable glucometer (Zyklusmed TD-4267), based on glucose oxidase enzymology, was chosen for its rapidity (<1 minute per reading), low cost (device ~$20 USD, strips ~$0.20 USD each), portability, and minimal sample preparation requirements. Although designed for capillary blood, glucometers have shown promise in non-blood applications such as fermentation monitoring and enzymatic kinetics when matrix interferences are controlled [e.g., educational assays for invertase/lactase; adapted personal glucose meters for broader diagnostics]. This study validates the glucometer specifically for GI activity by direct parallel comparison to HPLC, addressing the need for affordable alternatives in resource-limited laboratories and industrial settings.

2. Materials and Methods

2.1. Strain and Culture Conditions

To produce the glucose isomerase enzyme, a recombinant strain of *E. coli* BL21 harboring the synthesized gene was cultured in 15 modified M9 media formulations. These media contained three different carbon sources (glucose, lactose, and glycerol) and three distinct nitrogen sources (NH_4Cl, K_2NO_3, and yeast extract) under varying temperature conditions (37°C, 30°C, and 25°C), pH levels [6, 7, and 8], and a shaker speed of 180 rpm, with three replicates for each condition. Gene expression was induced by adding lactose. After 12 h of incubation, the cells were harvested by centrifugation at 4000 rpm for 10 min. The cell pellet was discarded, and the supernatant containing the secreted enzyme was collected and stored at refrigeration temperature until further use.

2.2. Enzyme Activity Assay

To assess enzymatic activity, 500 µL of the enzyme-containing supernatant was mixed with 500 µL of a reaction solution comprising 0.02 M glucose (and, in some trials, 0.4 M fructose), 10 mM $MgSO_4$, 1 mM $CoCl_2$, and 20 mM Tris–HCl buffer at pH 7. The reaction mixture contained 0.02 M glucose (corresponding to 20 µmol in the total 1 mL reaction volume). After vortexing, the reaction mixture was incubated on a hot plate at 80°C for 10 min and then immediately placed on ice to halt enzymatic activity.

The residual glucose concentration in the samples following glucose isomerase activity was evaluated using two methods, each accompanied by a standard glucose calibration curve within the operational range of the respective technique. For the glucometer method, standard glucose solutions of varying concentrations were prepared within the detection range of the glucometer (Zyklusmed TD-4267, Taiwan), and readings were recorded in mg/dL to construct a standard curve. To assess the specificity of the device, fructose solutions were also tested, confirming that the glucometer responded exclusively to glucose. The reaction mixtures derived from 15 different cultures were analyzed using a glucometer, and the reduction in glucose concentration compared to a negative control (500 µL buffer instead of enzyme supernatant) was used as an indicator of enzymatic performance. All experiments were conducted under controlled conditions at 37°C and pH 7 to simulate physiological conditions. In the HPLC method, samples were analyzed by injecting 20 µL into a Hypersil Ca^{2+} column using deionized water as the mobile phase. A glucose standard curve was also prepared within the operational range of the column and detector. Sugar identification was based on retention time compared to glucose and fructose standards in 15 replicates. As a positive control, commercial glucose isomerase (Sweetzyme®, Novozymes, USA) was used under identical conditions.

Glucose isomerase activity was quantified based on the amount of fructose produced or glucose consumed. One unit of enzymatic activity was defined as the amount of enzyme required to convert 1 µmol of glucose to fructose per minute under the specified assay conditions [16].

For the glucometer method (Zyklusmed TD-4267, Taiwan), reaction mixtures were cooled on ice, then 5–10 µL was directly applied to the test strip (no dilution required within the device's range of 20–600 mg/dL). The device provided readings in <5 seconds via amperometric detection based on glucose oxidase. Specificity was confirmed by testing standard fructose solutions (0–0.4 M), yielding no response (<10 mg/dL, below detection threshold). A negative control (buffer instead of enzyme) confirmed no glucose change, and a positive control (commercial Sweetzyme®) validated the setup. All measurements were performed in triplicate at room temperature (25°C), with the device calibrated per manufacturer instructions. Glucose consumption was calculated as: Δglucose = control reading – sample reading, converted to activity units as defined. This procedure ensures high reproducibility, requiring only standard glucometer operation without additional equipment. Glucometer readings (mg/dL) were converted to glucose concentration values using the device-specific calibration curve. Enzyme activity units were subsequently calculated based on Δglucose relative to the negative control.

2.3. Statistical Analysis

To evaluate the accuracy of the enzymatic activity measurements using the glucometer, parallel measurements were performed using HPLC. Simple linear regression was used to assess correlation, as it is appropriate for evaluating agreement between two continuous measurement methods. The independent t-test was selected to compare means, assuming normality (confirmed via Shapiro-Wilk test, $p > 0.05$ for all datasets) and equal variances (Levene's test, $p > 0.05$). Sample size: n=15 independent culture conditions (each in triplicate for HPLC; parallel glucometer readings), with additional regression on five replicates per temperature (25°C, 30°C, 37°C; total n=15 paired points for regression). Power analysis indicated >80% power to detect differences at $\alpha=0.05$. All data are presented as mean ± SD."Analyses were conducted using SPSS v.22.

3. Result and Discussion

Results obtained from HPLC analysis demonstrated that glucose isomerase produced by the recombinant *E. coli* BL21 strain effectively catalyzed the reversible isomerization of glucose ↔ fructose. All chromatograms exhibited three major peaks corresponding to lactose (13.6 min), glucose (21.1 min), and fructose (22.1 min). Changes in the peak areas indicated the partial conversion of the initial substrate (glucose or fructose) into the final product. It is important to note that the lactose peak originated from residual lactose in the culture medium, which was used both as a carbon source and as an inducer of protein expression and was unrelated to the enzymatic isomerization process. In the chromatograms of glucose-to-fructose isomerization reactions, a decrease in the glucose peak area accompanied by the emergence and increase of the fructose peak was observed. In Fig. 1, peak area analysis revealed that approximately 29.9% of the initial glucose was consumed and converted to fructose. Given an initial glucose concentration of 20 µmol, the amount of fructose produced was estimated to be approximately 5.98 µmol. Accordingly, the enzyme activity over the 10-minute reaction at 80°C was calculated as 0.598 U, and after normalization to the supernatant volume (0.5 mL), the specific activity was determined to be 1.2 U/mL. On a total scale, the enzyme activity extracted from 15 mL of culture medium was calculated to be 290 µmol/L/min, corresponding to the data reported in Table 1 for experiment number 23 (Fig. 1). In the second chromatogram, which represents the fructose-to-glucose isomerization reaction, a clear reduction in the fructose peak area and a corresponding increase in the glucose peak were observed. Calculations showed that approximately 22.5% of the initial fructose was consumed and converted to glucose. Based on an initial substrate concentration of 400 µmol of fructose, the amount of glucose produced was estimated to be approximately 90 µmol. Therefore, under these conditions, the enzyme activity was calculated as 9 µmol/min, and after normalization to the supernatant volume (0.5 mL), the specific activity was determined to be 18 U/mL. The total enzyme activity from 15 mL of culture medium was calculated to be 270 µmol/L/min, as reported for experiments 3 and 6 (Fig. 2). The cultivation conditions and enzymatic activity values measured by HPLC across 15 experiments, each with three replicates, are presented in Table 1.

To evaluate the accuracy and efficiency of enzymatic activity measurements using a glucometer, the results were compared with those obtained using HPLC. Data normality and homogeneity were verified prior to parametric tests. Data from five independent replicates at 25°C, 30°C, and 37°C showed a strong and statistically significant correlation between glucometer readings and HPLC results ($R^2 = 0.96$, $p = 0.0001$) (Fig. 3). Furthermore, an Independent t-test revealed no significant difference between the mean enzymatic activities measured using the glucometer and HPLC ($t = 0.28$, $p = 0.78$).

In this study, for the first time, a glucometer was employed as a simple, rapid, and cost-effective tool for measuring glucose isomerase (GI) activity in non-blood environments. This application is novel because previous glucometer-based enzymatic assays have mainly focused on hydrolysis reactions rather than isomerization, where selective glucose measurement is critical without interference from fructose. This method, based on monitoring changes in glucose concentration within the enzymatic reaction mixture, successfully quantified the conversion of glucose to fructose in various samples with acceptable accuracy and precision. The glucometer also proved effective in measuring the reverse reaction from fructose to glucose, demonstrating its versatility in bidirectional isomerization assays.

HPLC analysis confirmed that recombinant GI from *E. coli* BL21 efficiently catalyzed the interconversion of glucose and fructose in the presence of Mg^{2+} and Co^{2+} ions under optimal conditions (80°C, pH 7.6), highlighting the potential of this enzyme for industrial applications. A strong and statistically significant correlation between the glucometer and HPLC results ($R^2 = 0.96$, $p = 0.0001$) validated the reliability of the glucometer under controlled laboratory conditions. Moreover, no significant difference was observed between the mean enzymatic activities measured using the two methods, confirming the accuracy of this innovative approach.

From an industrial perspective, the use of glucometers offers several practical advantages. This significantly reduces the cost and complexity of enzyme activity assays, eliminating the need for expensive chromatographic equipment and highly trained personnel [5]. This can be especially beneficial for small- to medium-scale production facilities, pilot plants, and quality control laboratories in developing countries [17]. The rapid turnaround time and minimal sample preparation also enhance operational efficiency, allowing real-time monitoring and faster decision-making during fermentation and enzyme production processes [12].

Furthermore, the portability and ease of use of glucometers make them suitable for on-site testing in industrial environments, enabling decentralized enzyme screening and reducing dependence on centralized analytical laboratories. This flexibility can accelerate process optimization, reduce downtime, and improve overall productivity in enzyme-based manufacturing workflows [18]. These features are particularly valuable in resource-limited settings where access to advanced instrumentation may be restricted.

A comparison with previous studies revealed meaningful differences in glucometer performance depending on the sample type and environmental conditions. For instance, Otokunefor and Ogu (2018) reported significant discrepancies between glucometer readings and the glucose oxidase laboratory method in clinical samples affected by interfering factors [19]. In contrast, the present study achieved accurate results by tightly controlling the reaction conditions, such as temperature, pH, and metal ion concentration.

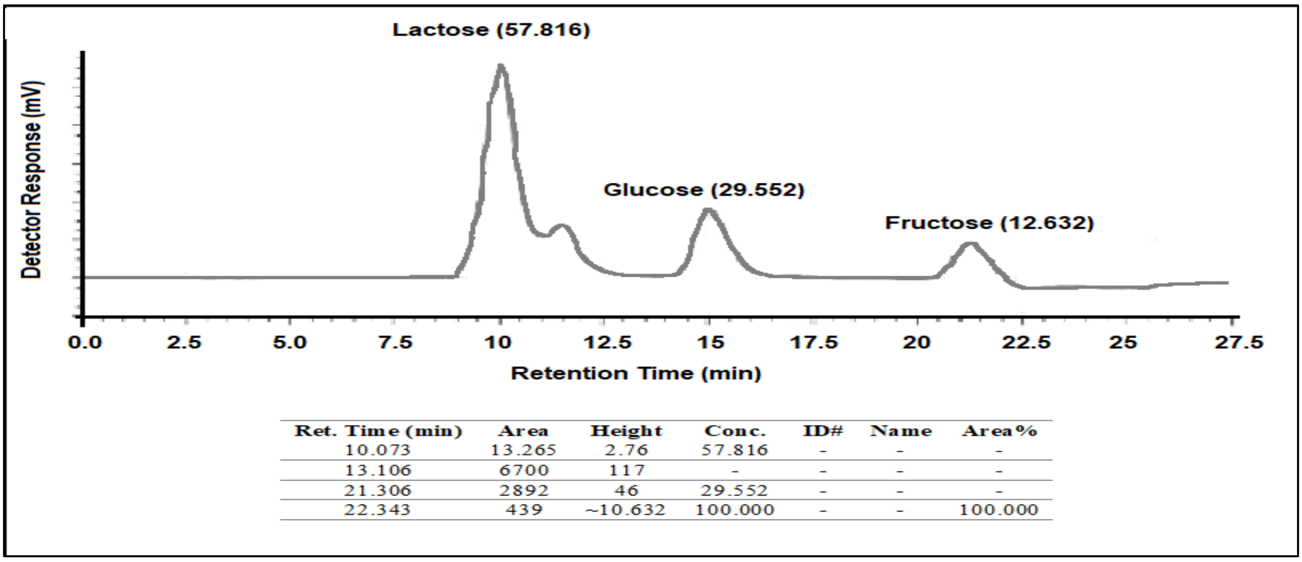
These findings are consistent with those of Darwin (2019), who demonstrated that the GlucoDr glucometer performed accurately in fermentation environments, showing high and significant correlation coefficients. Galant et al. (2015) emphasized that glucometer accuracy depends on the type of enzyme used, environmental conditions, and the presence of interfering compounds. The current study supports this view, showing that under optimized conditions, glucometers can be effectively utilized in industrial and biotechnological settings.

While the strong correlation ($R^2 = 0.96$) and lack of significant mean difference support glucometer reliability under our controlled conditions (pure reaction mixtures, known interferences minimized), potential limitations must be acknowledged. Glucometers are optimized for blood matrices; in non-blood samples like culture supernatants or industrial streams, factors such as pH extremes, high ionic strength, viscosity, or interfering reductants/antioxidants could affect accuracy (5). Variability (±10–20% typical for clinical glucometers) may be amplified without matrix-matched calibration. Further validation in complex industrial matrices (e.g., crude lysates, high-fructose feeds) is recommended before broad adoption. Our findings align with reports of glucometer utility in fermentation glucose tracking, where controlled conditions yield comparable results to chromatographic methods, but highlight the need for case-specific validation due to matrix effects.

In conclusion, although HPLC remains the gold standard for precise sugar analysis, glucometers can serve as effective complementary tools for rapid and low-cost glucose monitoring under suitable conditions. This innovation opens new avenues for developing enzymatic screening methods in educational, research, and industrial settings. Future studies are recommended to evaluate glucometer performance in more complex and large-scale environments to better define its application scope and unlock its full potential in enzyme-based production systems.

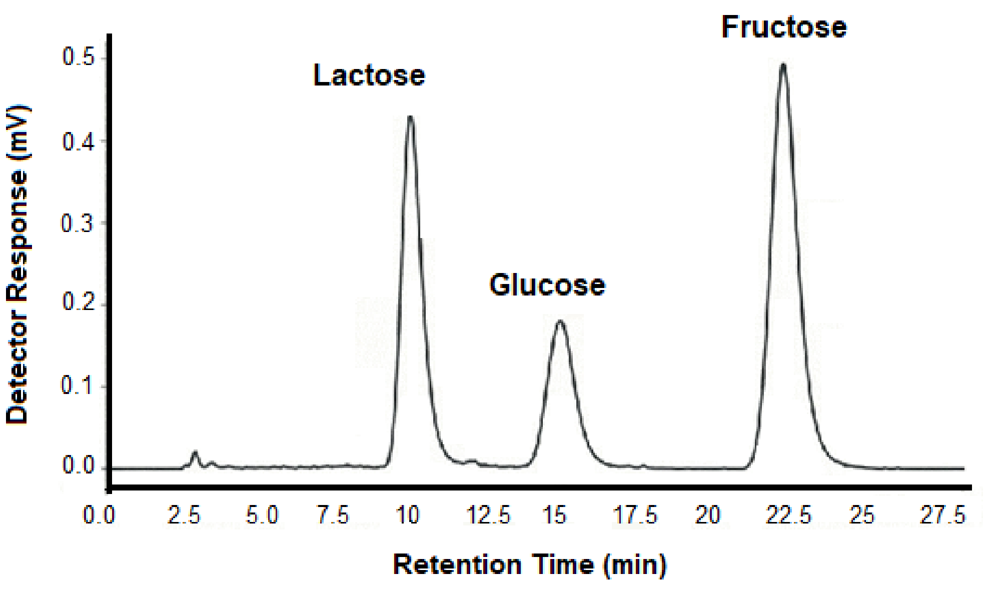
Table 1. Cultivation conditions and results of enzymatic activity measurement using glucometer and HPLC. D represents the carbon source, and E represents the nitrogen source. The first level of carbon source was glucose, the second was lactose, and the third was glycerol. The first level of nitrogen source was NH_4Cl, the second was K_2NO_3, and the third was yeast extract

Run	Temperature (°C)	pH	Induction time (h)	Carbone source [1=glucose, 2=lactose, 3=glycerol]	Nitrogen source [1=NH_4Cl, 2=K_2NO_3, 3=yeast extract]	Enzyme activity (micromol/L/minute)	
						Glucometer	HPLC
4	25	7	8	Level 1 of D	Level 2 of E	137.01	156.1494
22	25	7	4	Level 3 of D	Level 1 of E	1890.15	1834.101
23	25	7	0	Level 1 of D	Level 2 of E	302.85	292.039
24	25	8	4	Level 3 of D	Level 1 of E	1463.71	1511.2474
35	25	8	8	Level 1 of D	Level 1 of E	2340.28	2435.2232
3	30	6	4	Level 1 of D	Level 1 of E	255.47	285.5018
5	30	7	4	Level 2 of D	Level 1 of E	1345.26	1059.9044
14	30	8	0	Level 1 of D	Level 3 of E	942.1	1033.934
20	30	6	8	Level 1 of D	Level 2 of E	1416.33	1344.7102
34	30	7	4	Level 2 of D	Level 1 of E	889.63	971.6122
1	37	6	0	Level 2 of D	Level 1 of E	800.36	652.6984
2	37	8	4	Level 2 of D	Level 3 of E	1345.26	1009.9044
6	37	7	4	Level 1 of D	Level 3 of E	255.47	286.5018
26	37	8	4	Level 3 of D	Level 3 of E	871.44	800.5136
39	37	6	4	Level 3 of D	Level 2 of E	1398.56	1378.0064



Ret. Time (min)	Area	Height	Conc.	ID#	Name	Area%
10.073	13.265	2.76	57.816	-	-	-
13.106	6700	117	-	-	-	-
21.306	2892	46	29.552	-	-	-
22.343	439	~10.632	100.000	-	-	100.000

Figure 1. HPLC chromatogram corresponding to the glucose-to-fructose isomerization reaction. The supernatant sample from a 16-hour culture of recombinant *E. coli* BL21 in M9 medium (with lactose induction from the time of inoculation) was incubated with a reaction solution containing 0.02 M glucose, 10 mM $MgSO_4$, 1 mM $CoCl_2$, and 20 mM Tris–HCl buffer (pH 7.6) for 10 min at 80°C. Based on the observed decrease in glucose and increase in fructose, the final enzymatic activity was calculated to be 299 μmol/L/min



Peak	Component	Retention Time (min)	Area	Height	Area%
1	Lactose	10.173	16067	320	24.010
2	Glucose	15.347	11725	162	16.947
3	Fructose	21.747	38817	521	58.444
Totals			**66609**	**1026**	**100.000**

Figure 2. HPLC chromatogram of the fructose-to-glucose isomerization reaction. The supernatant sample, obtained under identical cultivation conditions, was incubated for 10 min at 80°C with a reaction solution containing 0.4 M fructose, 10 mM $MgSO_4$, 1 mM $CoCl_2$, and 20 mM Tris–HCl buffer (pH 7.6). Based on the fructose consumption and glucose formation, the final enzymatic activity was calculated to be 270 µmol/L/min

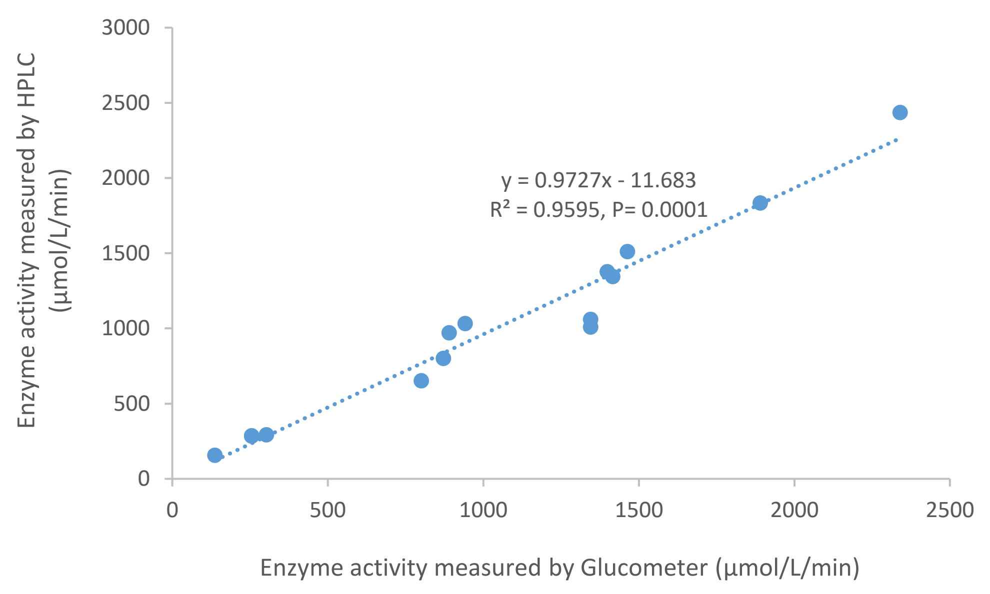


Figure 3. Simple linear regression curve illustrating the correlation between enzymatic activity measurements obtained using a glucometer and HPLC

4. Conclusion

The glucometer offers a promising rapid, low-cost complement to HPLC for GI activity screening in controlled laboratory settings and potentially pilot-scale monitoring, particularly where resources are limited. The glucometer provides a rapid, low-cost, and user-friendly alternative for measuring glucose isomerase activity with results comparable to HPLC. Practically, it enables high-throughput enzyme screening on benchtops and in pilot plants, allowing technicians to run dozens of assays per hour with minimal training. In process development and quality control, glucometers support near real-time monitoring of bioreactors and downstream processes, speeding optimization cycles and reducing downtime. In small- and medium-sized production facilities and remote or resource-limited settings, the device lowers capital and operational costs by replacing or complementing routine HPLC checks. In teaching laboratories and outreach programs, glucometers make hands-on enzyme kinetics experiments accessible and safe for students. With appropriate calibration and matrix validation, glucometers can therefore expand on-site decision-making, increase sampling frequency, and streamline workflows across biotechnology, food science, and educational applications.

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Authors Contribution

Saloomeh Shoaei Naeeni (Conceptualization [Equal], Data curation [Equal], Formal analysis [Lead], Investigation [Lead], Methodology [Equal], Visualization [Lead], Writing - original draft [Lead]), Bijan Bambai (Conceptualization [Equal], Formal analysis [Equal], Funding acquisition [Equal], Methodology [Equal], Supervision [Lead], Validation [Equal], Writing - review & editing [Lead]), Bagher Yakhchali (Supervision [Equal]), Parvaneh Saffarian (Supervision [Equal]), Zarrin Minuchehr (Supervision [Equal]).

Availability of data and materials

The data underlying this article will be shared on reasonable request to the corresponding author.

Conflict of interests

The author states that there is no conflict of interest.

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