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Quantitative Calcium Analysis in Human Nail Keratin Using LIBS Validated by ICP–MS: Implications for Diabetes Diagnostics

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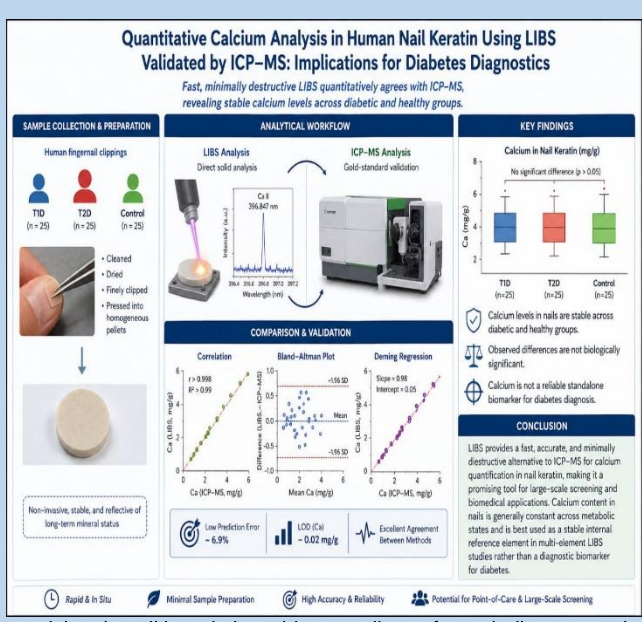
Abstract: The quantitative determination of calcium in human fingernail keratin by laser-induced breakdown spectroscopy (LIBS) is compared with the results of inductively coupled plasma mass spectrometry (ICP–MS) in the present study. In a controlled setting, 75 fingernail samples were collected from type 1 (T1D) and type 2 (T2D) diabetes patients and healthy controls (C). The calcium concentrations were then determined independently by both methods. The LIBS calibration model was developed using reference samples determined by ICP-MS and based on the Ca II emission line at 396.847 nm, which was selected due to its spectral stability and reduced self-absorption under optimal plasma conditions. The model showed a good linearity (R^2 = 0.958), low relative prediction error (~6.9%) and the detection limits suitable for biological keratin matrices. Quantitative validation with diabetic samples demonstrated strong analytical agreement between LIBS and ICP-MS. Bland-Altman plots, Deming regression and Pearson correlation analysis ($r > 0.998$) revealed small systematic bias. A moderate decrease in calcium concentration was observed in the type 1 diabetes group by LIBS; however, this trend did not remain statistically significant after Tukey HSD correction. ICP-MS analysis of nail Ca concentrations between the diabetic and healthy control groups did not show statistically significant differences. Rather than signifying a genuine biological change, this discrepancy could be a consequence of matrix-related sensitivity. These findings indicate that calcium in nail keratin is stable regardless of metabolic state and is not an effective biomarker for diagnosing diabetes. As a stable internal reference element for biomedical research based on multi-element LIBS, calcium may serve as a promising internal reference element for future multi-element LIBS studies.



Keywords: LIBS and ICP-MS techniques, human fingernails, diabetes mellitus, calcium quantification, analytical validation and biomarker stability

Introduction

An important macronutrient for bone mineralization, neuromuscular transmission, and intracellular signaling is calcium (Ca). Numerous pathological conditions, including osteoporosis, cardiovascular diseases, and metabolic diseases like diabetes mellitus, have been linked to dysregulation of calcium homeostasis(1-3). Because of their non-invasive sampling, long-term stability, and capacity to reflect cumulative metabolic or environmental exposure over extended periods of time, human fingernails have garnered increasing interest as a biological matrix in biomedical and environmental studies(4, 5).

Nails are relatively resistant to short-term physiological fluctuations, can be effectively cleaned to reduce external contamination(6, 7), and, given their slow growth rate, provide retrospective information spanning several months(8). For some elements, the properties of nail clippings are already well-defined, and they have been established as useful biomarkers(9).

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Therefore, Nails are a promising biological matrix for the biological monitoring of exposure to essential and toxic elements under normal and pathological conditions(10). Calcium is present in fingernails at relatively high concentrations compared with many other trace elements. These values make calcium an excellent candidate as a biomarker for long-term metabolic processes, as it is incorporated into keratin through continuous nail growth (11). Nail clippings are a potential biomarker for minerals, including calcium, assessing this element in nail clippings using two important techniques, laser-induced breakdown spectroscopy (LIBS) and inductively coupled plasma mass spectrometry (ICP–MS), is a relatively underexplored application. As an analytical method, LIBS has several important advantages over traditional methods such as ICP–MS. Although ICP–MS offer high accuracy, they require laborious sample digestion and long analysis times, which limits clinical production rates(12, 13).

However, for elemental analysis across a variety of sample matrices, including biological and environmental materials, LIBS has been widely acknowledged as a quick, localized, and minimally destructive method(14-16). The ability of LIBS to conduct direct multi-elemental analysis with little to no sample preparation is one of its main advantages, which makes it especially appropriate for point-of-care and real-time applications(17, 18).

LIBS has been used more frequently in environmental, agricultural, and medical research in recent years to quantify trace and macro elements. For instance, targeted studies have shown its potential in plant mineral assessment and clinical diagnostics(19-21), while comparative studies with XRF and AAS have shown its analytical reliability in food and biological samples(22, 23).However, the quantitative capabilities of LIBS analysis may be affected by matrix effects, signal fluctuations between snapshots, and the self-absorption effect, which may affect the linear correlation between emission intensity and element content(24, 25).

In the study by Almessiere et al., the researchers analyzed 71 human nail samples using LIBS and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) to determine the concentrations of mineral elements and their relationship with vitamin D deficiency. Potassium signals and vitamin D levels were found to be strongly correlated, and the ICP-AES results supported the LIBS results, showing LIBS as a viable rapid method for elemental analysis in nails (26). Jaramillo et al. have published a comprehensive review on the use of human nails as a biological matrix for the detection of disease biomarkers, emphasizing their potential as a non-invasive, time-stable material which reflects long-term biological changes.

The review elucidated the potential application of nails in studying metabolic, endocrine, oncological, and other diseases, emphasizing the diversity of analytical techniques employed and the need to standardize analytical methodologies and understand the mechanisms of biomarker incorporation within the nail structure before their widespread clinical adoption(27). The analytical accuracy of LIBS has been recently improved by calibration-free and internal normalization and cross-validation with reliable laboratory methods(28). Several studies have demonstrated the potential of LIBS technology for clinical diagnosis and biological studies of various biological samples as a response to kidney disorders and nutritional assessment(29-31). Sami et al. recently applied LIBS in calcium analysis of blood and serum in renal failure patients, as well as assessing the accuracy of LIBS versus XRF and ICP–MS in micronutrient determination in biologic and food samples(32).

For biomedical diagnostics, recent studies have investigated non-destructive spectroscopic methods like LIBS and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Using LIBS, Rithika et al. examined human fingernails and found that the concentrations of calcium, magnesium, and iron differed significantly between healthy people and those with diseases like diabetes and thyroid disorders. This suggests that nails could be used as non-invasive biomarker(33). Chan et al. supported the use of LA-ICP-MS in long-term elemental exposure assessment by demonstrating its accuracy for hair and nail analysis with recovery rates ranging from 97% to 105%(34). In a more recent study, Rehan et al. (2024) found that by combining LIBS with machine learning algorithms, diabetic patients could be identified with up to 96% accuracy using nail sample elemental compositions(35).The same group found that type 2 diabetic patients' nails had lower calcium, magnesium, and potassium intensities than those of healthy controls in a later study using LA-ICP-MS. Using principal component analysis (PCA) and an ensemble learning model integrating seven classifiers, they achieved excellent performance (96% accuracy, 96.7% sensitivity, and 99.9% specificity), reinforcing the feasibility of LIBS coupled with AI as a promising tool for rapid, non-invasive diabetes diagnosis(36).

Although these results demonstrate the potential of nail-based elemental analysis in biomedical research, many previous studies have little validation against high-precision quantitative reference techniques and instead rely on spectral intensity trends, multivariate statistics, or machine-learning classification models. Additionally, the robustness and reproducibility of quantitative results are still jeopardized by inconsistent sample preparation procedures and inadequate consideration of matrix-related effects, especially for major elements like calcium.

Therefore, the determination of calcium in human nails using LIBS should not be regarded as an attempt to establish a novel biomarker for diabetes mellitus, but rather as an analytical challenge that requires rigorous validation. In this regard, the current study intends to directly compare LIBS with ICP–MS as a reference method in order to systematically assess the analytical reliability of LIBS for quantitative calcium determination in human nail keratin. The impact of matrix effects, self-absorption behavior, and sample heterogeneity on LIBS signal stability are evaluated in particular, and the diagnostic limitations of calcium in differentiating between healthy individuals and patients with type 1 and type 2 diabetes mellitus are critically examined.

This work aims to define the proper role of calcium as a stable reference element for upcoming multi-element biomedical studies using LIBS and to standardize nail-based elemental analysis by elucidating the advantages and disadvantages of LIBS in this application.

Materials and Methods

Samples collection and Preparation

Seventy-five human fingernail samples were collected from volunteers aged 25 to 65 years. The patient group was matched for characteristics such as age, sex, place of residence, smoking, and dietary habits, as shown in table 1. A control group of healthy individuals was also formed and screened to exclude the disease under study. This allowed for the identification of the main components of the nails and ensured a fair comparison.

Participants were divided into three clinically identical groups: a control group of healthy individuals C (n = 25), individuals with type 1 diabetes T1D (n = 25), and individuals with type 2 diabetes T2D (n = 25). All participants provided informed consent before sample collection. The study was approved by the Institutional Review Committee of the College of Science, Mustansiriyah University according to the Declaration of Helsinki, reference number BCSMU/0125/0007Ph in January 2025.

locations to reduce crater overlap and maintain sample integrity. At each location several laser shots were collected and averaged in order to increase the reproducibility of the signal and decrease the pulse-to-pulse fluctuations. This approach is a trade-off between spatial coverage and repeatability of the experiment but more sampling points could better represent the sample heterogeneity. The analytical line of the 396.847 nm Ca II was selected due to the strong emission intensity and stable signal response under the plasma conditions used. The relatively low laser fluence was chosen to minimize the possible self-absorption effects associated with this resonance line. Linearity of calibration curve was a confirmation of the correct choice.

LIBS Method

The human fingernail clippings were rinsed with deionized water, immersed in 70% ethanol to remove surface impurities and dried at 60°C. A portion of each sample was used to make a pellet for LIBS analysis. Samples were cut and ground in a mixing mill and then dried to remove any remaining moisture. The mixture was then homogenized thoroughly after addition of 2% w/w non-metallic binder (polyvinyl alcohol or PVA). Pellets were produced by compressing 8 mm diameter blocks of the mixture at 10 tons for 120 sec in a hydraulic press. The resulting discs were 5 mm thick with smooth and stable surfaces. LIBS analysis was performed on the surface of each pellet to obtain ten spectra that were averaged. Figure 1. Typical samples prepared from human nail clippings for LIBS analysis.

Polyvinyl Alcohol (PVA) of analytical grade purity was used as binder for preparation of pellets. Blank PVA pellets were analyzed under the same LIBS conditions to exclude any possible contribution of calcium from the binder. Blank samples did not show any detectable calcium signal indicating that the calcium content measured was not contributed from PVA.

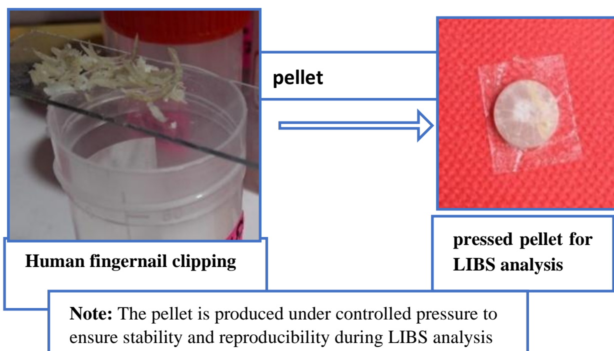


Figure 1: A typical set of nail clippers during cleaning and after pressing and forming pellets.

In the LIBS technique, a high-energy laser pulse creates a plasma on the surface of the sample, which results in the rapid evaporation and ionization of the surface layer. As the plasma cools, light is emitted with spectral lines characteristic of the elements and collected by a spectrometer for qualitative and quantitative analysis. A time-gate system is used to reduce background noise in the early stages of the plasma because the signal resolution is dependent on factors such as matrix effects, surface roughness, and laser power(37-39).

LIBS measurements were conducted using a Q-switched Nd:YAG laser operated at the fundamental wavelength of 1064 nm, with a pulse energy of 80 mJ, repetition rate of 4 Hz, and pulse duration of 10 ns. The laser beam was focused onto the nail pellet surface using a plano-convex quartz lens with a focal length of 100 mm. The laser spot diameter on the sample surface was approximately 0.93 mm, corresponding to a spot area of about 6.82×10^{-3} cm². Accordingly, the laser fluence was calculated as $F = E/A = 0.080 \text{ J} / 6.82 \times 10^{-3} \text{ cm}^2 = 11.738 \text{ J cm}^{-2}$. Reporting the spot size and fluence improves the reproducibility of the ablation conditions and facilitates comparison with other LIBS studies. The plasma emission was captured at an angle of 45° with respect to the incident beam and guided to a fiber cable coupled to a high-resolution spectrometer (Andor Shamrock 500i) combined with an intensified charge-coupled device (ICCD) camera (Andor iStar DH334T). The main components of the LIBS system arrangement are shown in Figure 2.

The delay time and gate width of the ICCD detector, which achieved the best signal-to-noise ratio, were set to 1.0 μs and 10 μs, respectively. System calibration and wavelength accuracy were verified using a standard mercury-argon lamp before each measurement session. Considering the self-absorption effects typical of keratinous biological samples as well as plasma interactions with the organic matrix, the spectral emission lines for quantitative analysis using LIBS were carefully chosen. The complex organic matrix and comparatively high calcium content of human fingernails can cause significant self-absorption and spectral line broadening, especially at strong resonant transitions like CaII (393.37 nm) and CaI (422.67 nm).

In order to reduce these effects, data acquisition parameters of LIBS including gate delay and integration time were optimized to suppress continuous emission in early plasma phases and promote atomic emission conditions with high signal-to-background ratio. In this way, the calcium emission lines with higher stability, lower self-absorption and better linearity with reference concentrations were preferred to ensure the reliability and reproducibility of the quantitative analysis.

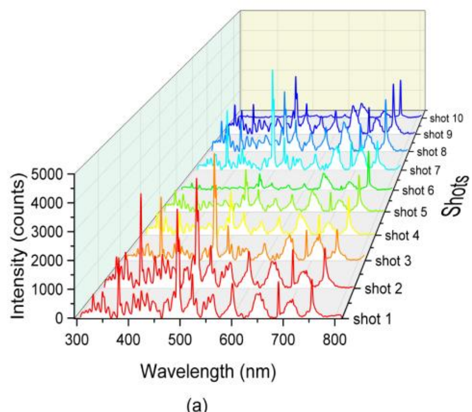
The calcium emission lines at 393.366, 396.846, 422.672, and 501.997 nm were analyzed in detail by qualitative analysis and the Ca II line at 396.847 nm was chosen as the quantitative analytical line. This line was clear and consistent in both healthy and diseased samples, with limited spectral overlap and stable response, appropriate for quantitative applications.

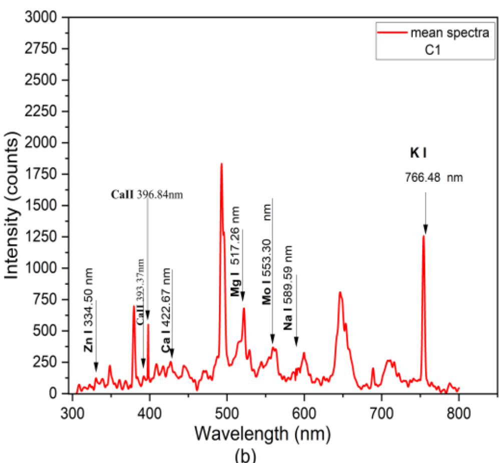
Each sample had two different spots cut out and each spot was irradiated by five laser pulses (five × two = ten pulses per sample). To reduce the spatial variability and to improve the reproducibility of the measurement the intensity of the Ca II line (I 396) averaged over the ten spectra was taken as the representation of the sample (Figure 3). The analysis of human fingernails was subjected to rigorous methodological controls to ensure that spectral signals reflect the targeted pathological condition and to minimize the effects of matrix and non-pathological individual variation. These controls included specific inclusion and exclusion criteria for constructing the statistical modeling database, excluding spectra with low signal-to-noise ratios, pulse instability, or weak atomic linearity, as well as samples exhibiting structural abnormalities or permanent

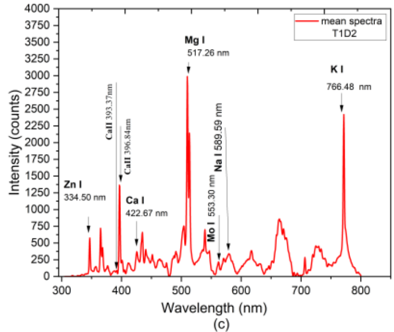
cosmetic coatings that could affect the laser's interaction with the surface.

Experimental setup for LIBS analysis of nail samples using an Nd: YAG laser system. The laser -induced plasma emission is collected via an optical fiber and analyzed by a high-resolution spectrometer. The system is controlled by dedicated software.

Figure 2: The main components of the LIBS system.







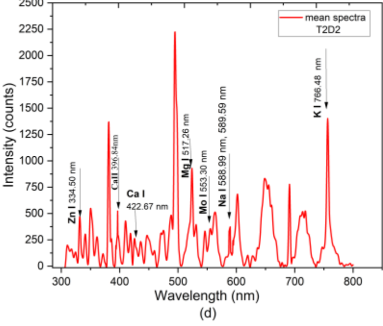


Figure 3: LIBS plasma emission spectrum of normal human nail sample (a) The ten individual spectra sample C1, (b) the average spectra C1, (c) the average spectra T1D2, and (d) the average spectra T2D2

ICP–MS method

The remainder of each sample was used to determine the calcium level using an ICP–MS (Thermo Fisher Scientific Neptune Plus), as shown in figure 4. Approximately 50 mg of each nail in the sample was digested using a closed-loop microwave digestion system with high-purity HNO_3 (65%) and H_2O_2 (30%) at concentrations of 2 mL and 1 mL, respectively. The digestion was performed at 800 W microwave power for 20 minutes at 180°C. The solutions were cooled and diluted to 25 mL in ultrapure deionized water (18.2 MΩ·cm) after digestion. Approved calcium standards (Merck, 1000 ppm) were used for titration after dilution in the same acidic matrix. Validation of the method demonstrated an analytical accuracy of ±2% and a recovery rate of 96% to 104%.

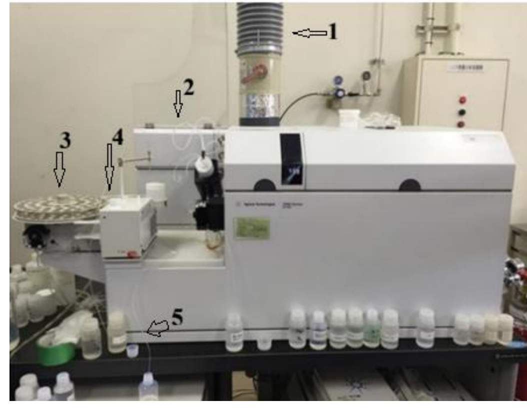


Figure 4: ICP–MS instrument used for quantitative calcium determination, and its main components are: **(1)**Exhaust system, **(2)**Peristaltic pump,**(3)**Autosampler,**(4)**Spray chamber,**(5)**Sample and standard solutions.

Statistical analysis

To perform calcium concentration comparisons, SPSS software (version 29.0, IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Data are presented as mean ± standard error (SE). Normality was assessed using the Shapiro–Wilk test and homogeneity of variances was evaluated before group comparisons. Differences among study groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for multiple comparisons. A significance level of $P \leq 0.05$ was considered statistically significant.

Sample-size justification and effect-size assessment were added to clarify the statistical strength of the study. The cohort size (n = 25 per group; total n = 75) was selected as a feasible pilot-scale sample for analytical validation of LIBS against ICP–MS rather than for definitive clinical biomarker qualification. The effect size of the LIBS comparison between the control and type 1 diabetes groups, was moderate (Cohen's d ≈ 0.66) with an estimated post-hoc power of 0.63 at α = 0.05 for n = 25 per group. This sample size would yield only approximately 80% statistical power for large effect size (d ≥ 0.80). By contrast, the ICP–MS comparisons were marked by small effect sizes (Cohen's d ≈ 0.10–0.17), consistent with the interpretation that nail calcium does not show a robust disease-related shift. Therefore, the present results should be interpreted primarily as analytical validation data, while larger multicenter cohorts are required for biomarker validation.

Power Analysis

To further assess the statistical sensitivity of the study, a post-hoc power analysis was conducted using the observed effect sizes and sample size (n = 25 per group). The results are summarized in Table 4. As shown in Table 4, the observed statistical power was adequate only for moderate-to-large effects. Therefore, while the current sample size was sufficient for analytical validation purposes, larger cohorts may be required to detect subtle biological differences in calcium concentrations among diabetic and healthy individuals.

Results and Discussion

A total of 75 human fingernail samples were analyzed, equally distributed among the control (C), type 1 diabetes (T1D), and type 2 diabetes (T2D) groups (n = 25 per group). The samples were collected from participants attending the National Center for Diabetes Treatment and Research, Mustansiriyah University, Baghdad, Iraq. To assess the comparability of the study population and identify potential confounding factors, baseline demographic characteristics were evaluated and are summarized in Table 1. No significant difference was observed in sex distribution among the three groups (P = 0.477), indicating a comparable representation of males and females. In contrast, age distribution differed significantly between groups (P = 0.0001), with the T2D group showing a higher proportion of participants aged ≥45 years, consistent with the established epidemiology of type 2 diabetes mellitus(40). Smoking status showed no statistically significant difference among groups (P = 0.911). These baseline characteristics were considered during the interpretation of calcium measurements obtained by LIBS and ICP–MS in order to minimize potential confounding effects and improve

Table 1: Baseline characteristics of the study participants.

Factor		**Control No. (%)**	**Diabetes type 2 No. (%)**	**Diabetes type 1 No. (%)**	**P-value**
Gender	Male	11 (44.00%)	13 (52.00%)	14 (56.00%)	0.477 NS
	Female	14 (56.00%)	12 (48.00%)	11 (44.00%)	
Age groups (year)	10-24 yr.	7 (28.00%)	1 (4.00%)	8 (32.00%)	0.0001 **
	25-44 yr.	6 (24.00%)	1 (4.00%)	4 (16.00%)	
	≥45 yr.	12 (48.00%)	23 (92.00%)	13 (52.00%)	
Smoking	Yes	5 (20.00%)	4 (16.00%)	4 (16.00%)	0.911 NS
	No	20 (80.00%)	21 (84.00%)	21 (84.00%)	
Total		25	25	25	--

**** (P≤0.01), NS: Non-Significant.**

Calibrating between LIBS and ICP–MS

Real reference samples were used, defined by ICP–MS (i.e., the same sample measured by ICP–MS and used to construct the LIBS calibration model). A calibration model was established based on the relationship between the LIBS intensity of the Ca II emission line at 396.847 nm (I_396) and ICP–MS for the corresponding calcium concentration (C) using the 25 specimens from the control group. For all LIBS intensity data, the mean spectra were calculated in order to minimize spatial heterogeneity and to consider signal repeatability.

The high coefficient of determination demonstrates good linearity between the measured LIBS signal and the calcium concentration in the tested range (about 500–3000 ppm). The strong correlation indicates a strong relationship between the intensity of LIBS emission at Ca II 396.847 nm and changes in calcium content present within nail keratin. The slope indicates the average increase of approximately 61 counts in the LIBS signal for each 100-ppm change in calcium concentration, demonstrating good analytical sensitivity. Residuals were randomly scattered around 0 with no apparent systematic bias, indicating that the linear model is appropriate.

The standard error of estimate (SEE ≈ 0.08 ppm) and the relative prediction error (RPE ≈ 6.9%) were acceptable for an analytical method. The detection limit (22 ppm) and the quantification limit (73 ppm) show that the method is sufficiently sensitive for the trace analysis of calcium in biological keratin matrices when calculated from those parameters. The calibration established in figure 5 was then applied to the diabetic samples (T1D and T2D) to estimate the calcium levels from the LIBS intensities and to independently validate the LIBS measurements against the ICP–MS results.

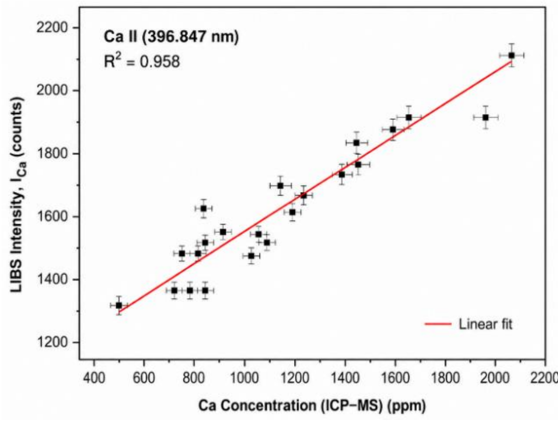


Figure 5: Linear calibration curve for control group plotting LIBS intensity (I_396, counts) vs calcium concentration (C, ppm). The shaded area is the confidence interval of 95%. The linear regression line is fairly fitted.

Validation Strategy

The LIBS calibration model was developed using the 25 control samples for which calcium concentrations were independently determined by ICP–MS. The resulting calibration equation was subsequently applied to the diabetic samples (25 T1D and 25 T2D) as an independent external validation set. Method performance was evaluated using Pearson correlation analysis, Deming regression, Bland–Altman agreement analysis, relative prediction error (RPE), and recovery assessment to ensure the analytical reliability of LIBS measurements.

Validation in Diabetic Samples (T1D and T2D)

The calibration model was tested quantitatively on type 1 and type 2 diabetic patients' nails. The method was developed and calcium concentrations were estimated by laser-induced optical emission spectroscopy (LIBS) of type 1 and type 2 diabetic patients and compared to reference measurements using ICP–MS. Table 2 shows calcium concentrations of fifty volunteers of two diabetic groups using ICP-MS and LIBS techniques. Some of the errors were relatively large for some of the samples (e.g., 16%). These can be attributed to the heterogeneity of the local biological matrices, the variation in surface roughness, matrix effects and variability in excision. Such factors are inherent to nail keratin analysis and were minimized by averaging multiple readings.

Table 3 summarizes calcium concentrations in nail samples for each study group measured by ICP–MS and LIBS. Group differences were evaluated according to the statistical strategy described above, and the pairwise comparisons are reported with their corresponding P-values. ICP–MS did not show statistically significant differences in mean calcium concentration among the control, type 1 diabetes, and type 2 diabetes groups ($P = 0.8397$). For LIBS measurements, the T1D group showed lower mean calcium concentrations than the control group. However, after applying Tukey's HSD correction for multiple comparisons, the difference did not reach statistical significance ($P = 0.074$). Similarly, no significant differences were observed between the control and T2D groups. These findings are consistent with the ICP–MS results, which also showed no statistically significant differences among the study groups. These findings are illustrated in Figure 6.

Uppercase and lowercase letters indicate differences between groups: uppercase letters compare columns within the same analytical method, and lowercase letters compare rows between the two methods for the same group. Although LIBS measurements showed a tendency toward lower calcium concentrations in the T1D group, this trend did not remain statistically significant after correction for multiple comparisons using Tukey's HSD test. The absence of statistically significant differences in both LIBS and ICP–MS measurements strengthens the conclusion that calcium concentration in nail keratin is largely stable across the investigated diabetic and non-diabetic populations.

Although numerical differences were observed between LIBS and ICP–MS measurements in the type 1 diabetes group, these differences did not remain statistically significant after Tukey HSD correction. The Bland–Altman analysis shown in Figure 7 revealed a slight mean bias with narrow limits of agreement. There is no indication of systematic or proportional bias across the measured concentration range, and the narrow 95% limits of agreement demonstrate good analytical consistency between the two techniques. Deming regression analysis, as shown in Figure 8, confirmed that the regression values were close to one. High correlation coefficients ($r \geq 0.998$), near-one regression slopes, and near-zero intersection points demonstrated strong analytical agreement, confirming the absence of relative measurement error between the two analytical methods. The data demonstrate that the observed differences do not indicate systematic or relative measurement errors, but are likely related to the baseline material's effects in the nail samples from type 1 diabetic patients.

Table 2: Calcium concentration using ICP–MS and LIBS techniques for T1D and T2D diabetic patients, using calibration between the two techniques.

T2D	C-ICP–MS (ppm)	C-LIBS (ppm)	error %	T1D	C-ICP–MS (ppm)	C-LIBS (ppm)	error %
T2D1	1270	1228	3.5	T1D1	720	700	2.7
T2D2	1910	1923	0.8	T1D2	1420	1420	0.3
T2D3	1100	1030	6.6	T1D3	540	530	2.5
T2D4	1600	1560	2.3	T1D4	750	720	4.5
T2D5	200	200	1.5	T1D5	1304	1320	1.2
T2D6	2620	2631	0.6	T1D6	1090	1060	3.1
T2D7	1280	1275	0.4	T1D7	1230	1210	1.3
T2D8	1120	1150	3.0	T1D8	1530	1510	1.5
T2D9	1270	1260	1.2	T1D9	2070	2120	2.0
T2D10	1080	1030	5.0	T1D10	1270	1170	8.5
T2D11	540	450	16.1	T1D11	2370	2340	1.2
T2D12	1480	1460	1.8	T1D12	1690	1640	2.8
T2D13	1080	1067	1.5	T1D13	1390	1370	1.2
T2D14	970	950	2.5	T1D14	1140	1130	0.2
T2D15	1480	1460	1.0	T1D15	1230	1220	1.1
T2D16	1050	1035	1.9	T1D16	980	930	5.0
T2D17	1310	1300	0.8	T1D17	980	950	2.5
T2D18	1210	1190	1.9	T1D18	1130	1130	0.1
T2D19	1050	1030	2.6	T1D19	1130	1030	8.8

T2D20	900	860	4.0	T1D20	2370	2340	1.1
T2D21	1350	1330	1.2	T1D21	580	570	2.0
T2D22	980	970	1.3	T1D22	600	600	0.5
T2D23	1960	1930	1.5	T1D23	980	950	3.0
T2D24	1400	1380	1.5	T1D24	1010	1005	0.9
T2D25	900	870	3.5	T1D25	450	440	2.4

Table 3: Comparison of Calcium Concentrations Among Study Groups and Analytical Techniques

Group	ICP–MS (ppm) Mean ± SE	LIBS (ppm) Mean ± SE	Tukey HSD (P-value)
Control	1290 ± 112 A	1290 ± 115 A	NS
Diabetes Type 2	1240 ± 93 A	1080 ± 119 A	NS
Diabetes Type 1	1200 ± 103 A	930 ± 102 A	NS
ANOVA (P-value)	0.839	0.090	---

Table 4 :Post-hoc Power Analysis Results

Comparison	Analytical Technique	Cohen's d	Statistical Power
Control vs T1D	LIBS	0.66	0.63
Control vs T2D	LIBS	0.35	Low
Control vs T1D	ICP–MS	0.16	Low
Control vs T2D	ICP–MS	0.08	Very Low

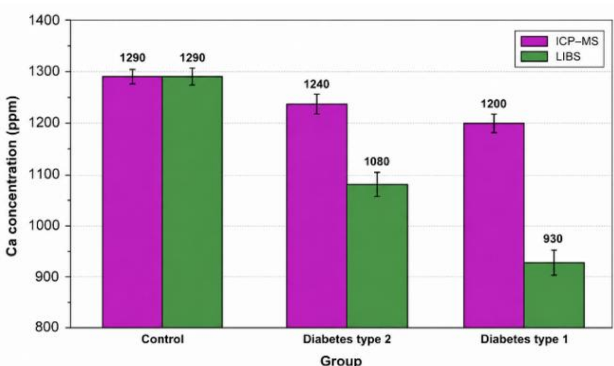


Figure 6: Comparison between different groups and technical type.

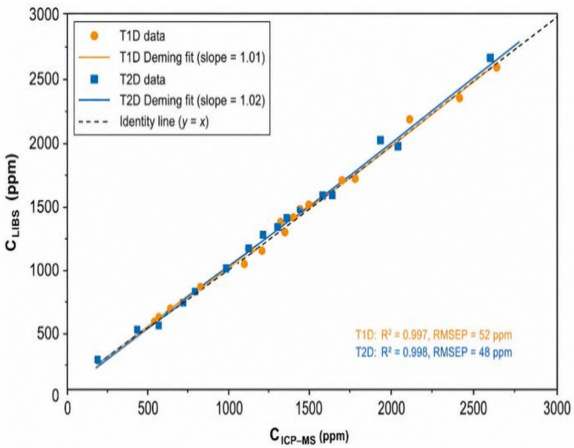


Figure 7: Bland-Altman analysis evaluating the consistency of calcium concentrations in T1D and T2D nail samples as determined by LIBS and ICP–MS.

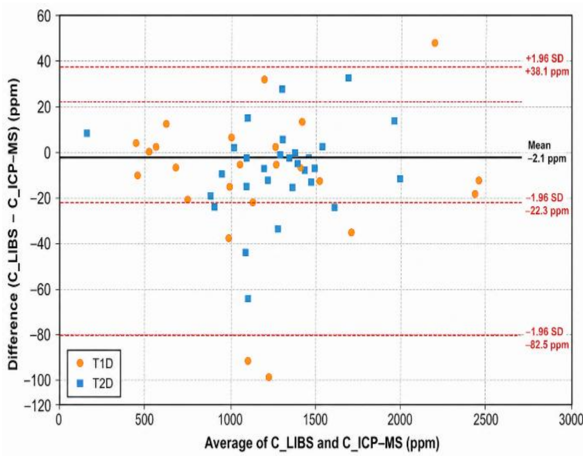


Figure 8: Deming regression analysis of the T1D and T2D groups' calcium concentrations as determined by LIBS versus ICP–MS.

Interpretation and Discussion

Calcium incorporation into keratinized nail tissue appears to be relatively stable across various metabolic states, as evidenced by the lack of statistically significant differences in calcium concentrations between healthy and diabetic nail samples. This finding supports the notion that calcium as a major structural component of keratin is less susceptible to metabolic disorders associated with diabetes than trace elements (41).

This interpretation is in line with evidence from systematic reviews that diabetes mellitus primarily affects the homeostasis of trace elements such as iron, copper and zinc with major elements such as calcium being relatively unaffected from a biochemical perspective. Therefore, the small numerical decreases seen in the diabetic groups are more likely to be due to secondary factors, such as nutritional status or altered mineral metabolism, rather than a direct pathophysiologic effect of diabetes.

The strong analytical agreement of LIBS with ICP–MS measurements based on Bland-Altman analysis and Deming regression further confirms the robustness of LIBS to quantify calcium in nail tissue, even in the presence of minimal biological variation. The relatively small standard deviations within groups also suggest that sampling was consistent and spatial averaging was efficient, which can be achieved by averaging multiple laser shots per sample.

The apparent discrepancy between LIBS and ICP–MS can be explained by the fundamentally different sampling mechanisms of the two techniques. ICP–MS measures the total calcium concentration after complete digestion of the nail material. LIBS interrogates a small local ablation volume and is therefore sensitive to surface morphology, keratin density, pellet compactness and local mineral distribution. In biological keratin matrices, these factors may alter the plasma temperature, electron density, ablated mass and emission efficiency without the associated change in the total calcium concentration. Thus, a LIBS-only difference cannot be considered sufficient evidence

for a diabetes-specific calcium biomarker without independent confirmation by a bulk reference method and larger validation cohorts.

Overall, the results indicated that the type of diabetes does not have a significant impact on nail calcium concentrations. The stability of Ca suggests that it can be used as an internal normalizer for the quantitative assessment of other trace elements in future multi-element or diagnostic nail studies.

Limitations

This study has a few limitations. First, the relatively small sample size and the single-center setting of the study may limit the generalizability of the findings. The post-hoc power assessment (Table 4) showed that the present sample size is largely sufficient to detect large effects, whereas small-to-moderate biomarker effects may require larger cohorts. Secondly, the LIBS calibration model was developed only on the control group, providing a stable baseline matrix, but may not fully capture the biological variability of diabetic nail samples. Third, the study was concentrated on calcium, while other trace elements or multi-element ratios may have more diagnostic value in diabetes. Thus, larger calibration and external validation datasets with both healthy and diabetic samples should be included in future multicenter studies, and prospective power analysis should be conducted before any clinical biomarker claims are made.

Conclusion

This work demonstrates that LIBS can measure calcium in human nail keratin with high linearity, precision and low systematic bias when calibrated against ICP–MS reference measurements. As a quick and minimally damaging method for nail-based elemental analysis, LIBS's analytical validity is confirmed by the high agreement between LIBS and ICP–MS in both healthy and diabetic samples. As determined by the reference method ICP–MS, calcium concentrations in fingernails do not differ statistically significantly between healthy individuals and patients with type 1 or type 2 diabetes, suggesting that nail calcium is largely insensitive to diabetic metabolic status.

The absence of statistically significant differences after Tukey HSD correction further supports the conclusion that calcium concentration in nail keratin is not a reliable standalone biomarker for diabetes discrimination.

Calcium should not be regarded as a primary diagnostic biomarker for diabetes, as evidenced by the limited discrimination seen by LIBS in type 1 diabetes samples, which is ascribed to analytical sensitivity rather than a strong pathological signature. Future multi-element LIBS studies aiming to identify more metabolically responsive trace elements may find calcium in nail keratin to be a suitable internal normalization or reference element due to its relative stability. Prior to suggesting clinical or diagnostic applications, reference-based validation is essential, and this work lays out a standardized analytical framework for assessing LIBS performance in biological keratin matrices.

Disclosure Statement

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the College of Science, Mustansiriyah University, Baghdad, Iraq, under reference number BCSMU/0125/0007Ph (January 2025).

All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Written informed consent was obtained from all participants prior to sample collection and analysis.

Consent for publication

Not applicable

Availability of data and materials

The raw data required to reproduce these findings are available in the body and figures of this manuscript. Additional data related to this study are available from the corresponding author upon reasonable request.

Author's contribution

Sura Allawi Obaid contributed to sample collection, experimental work, LIBS measurements, data acquisition, and manuscript drafting.

Muayyed Jabar Zoory contributed to study conception and design, supervision of the analytical methodology, interpretation and discussion of the results, statistical analysis, and critical scientific revision of the manuscript.

Haidar J. Mohamad contributed to ICP–MS measurements, analytical validation, and interpretation of the comparative results.

All authors reviewed the results and approved the final version of the manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Dissertation Statement

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