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LABORATORY VALIDATION OF A COST-EFFECTIVE WALKLEY–BLACK TITRIMETRIC METHOD FOR THE DETERMINATION OF SOIL ORGANIC CARBON

Aleksandar Piperevsk[1], *Biljana Balabanova*[1]

Abstract: Soil organic carbon (SOC) is a key indicator of soil health, fertility, and sustainability, influencing nutrient cycling, soil structure, and biological activity. This study validated a classical Walkley–Black titrimetric method for routine SOC determination under laboratory conditions. The method is based on wet oxidation of organic carbon using potassium dichromate in concentrated sulfuric acid, followed by back-titration of residual dichromate with standardized ferrous sulfate using diphenylamine as an indicator. Method validation included assessment of linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Calibration was performed using glucose standards covering 0.1–3.0% organic carbon. A BIPEA certified reference soil was used to ensure traceability and evaluate trueness. Excellent linearity was obtained (R^2 = 0.999). Precision showed relative standard deviation values below 5%, while accuracy assessed by standard addition yielded recoveries of 99.3–100%, indicating negligible matrix effects. The calculated LOD and LOQ were 0.01% and 0.04% OC, confirming that the method is sufficiently sensitive, reliable, and cost-effective for routine SOC analysis in agricultural and environmental soils.

Keywords: soil organic carbon, Walkley - Black titration, soil analysis, method validation.

Introduction

Soil organic carbon (SOC) is a fundamental component of soil organic matter and a key indicator of soil health, fertility, and ecosystem services (Nelson & Sommers, 1996; Lal, 2004; Don et al., 2011; Griscom et al., 2017). SOC strongly influences nutrient cycling, aggregation, water retention, and biological activity, while its accurate determination is essential for evaluating soil quality and monitoring changes driven by land management, land use change, and climate variability (Pribyl, 2010; Lehmann & Kleber, 2015). Regular

[1]Faculty of Agriculture, Goce Delcev University, Krste Misirkov 10-A, 2000 Stip, Republic N. Macedonia (apiperevski@yahoo.com; biljana.balabanova@ugd.edu.mk)

SOC monitoring supports sustainable agriculture, carbon sequestration strategies, and climate change mitigation through carbon budgeting (Blair et al., 1995; Post et al., 2001; Six et al., 2002). Several analytical methods are available for SOC determination. Dry combustion using elemental analyzers or TOC analyzers is considered a reference approach due to high precision and complete oxidation (Nelson & Sommers, 1996), but it requires expensive instrumentation, technical expertise, and regular maintenance (Balabanova et al., 2024). In contrast, wet oxidation methods, particularly the Walkley–Black procedure, remain widely applied in routine laboratories because of their simplicity, accessibility, and cost-effectiveness (Nelson & Sommers, 1996; Yeomans & Bremner, 1988; Chan et al., 2007). The method is based on partial oxidation of organic carbon with potassium dichromate in concentrated sulfuric acid, followed by back-titration of residual dichromate with ferrous sulfate. The redox indicator diphenylamine is used to detect the endpoint through a color change from violet-blue to green. Despite its wide use, the Walkley–Black method may be affected by incomplete oxidation of resistant organic carbon fractions and soil matrix effects, requiring laboratory-specific validation to ensure reliability. Therefore, this study presents a full validation of the Walkley–Black titrimetric method using glucose calibration standards (0.1–3.0% OC) and BIPEA certified reference soil material, evaluating linearity, precision, accuracy, LOD, LOQ, repeatability, and intermediate precision. The aim of this study was to evaluate and validate the analytical performance of the Walkley–Black titrimetric method and confirm its suitability for routine SOC determination in agricultural soils as a rapid and cost-effective procedure.

Materials and methods

Analytical Procedure (Walkley–Black titrimetric determination and blank)

Soil organic carbon (SOC) was determined by the classical Walkley–Black wet oxidation titrimetric method (Walkley & Black, 1934). For each determination, 0.50 g of air-dried and < 2 mm sieved soil material (including the BIPEA certified reference soil) was weighed into a 500 mL Erlenmeyer flask. Briefly, 10.0 mL of 1 N potassium dichromate solution (0.167 M $K_2Cr_2O_7$) was added, followed by rapid addition of 10.0 mL concentrated H_2SO_4. The mixture was immediately swirled and allowed to react for 30 min at ambient temperature. After oxidation, the solution was diluted with 200 mL distilled water, followed by addition of 10.0 mL H_3PO_4 and 2.0 mL diphenylamine indicator. Excess unreduced dichromate was back-titrated with standardized $FeSO_4 \cdot 7H_2O$ until the endpoint color

changed from violet-blue to green. A reagent blank was prepared in parallel by following the same procedure without soil material. Blank and sample titration volumes were recorded, and SOC (%) was calculated according to the Walkley–Black stoichiometric equation (Eq. 1), applying the conventional oxidation correction factor (1.33).

$$OC\% = \frac{(Vb - Va) \cdot N \cdot 0.003 \cdot 1.33 \cdot 100}{mass\ of\ soil\ g} \quad (1)$$

Where:

- (*Vb*) = volume (mL) of $FeSO_4$ used for blank titration
- (*Va*) = volume (mL) of $FeSO_4$ used for sample titration
- (*N*) = normality of $FeSO_4$
- 0.003 = g of C oxidized by 1 mL of 1 N $K_2Cr_2O_7$
- 1.33 = correction factor (based on ~77% oxidation efficiency)
- (m) = mass of soil sample (g)

Preparation of Calibration Standards

Glucose standards corresponding to 0.1 – 3.0% OC equivalents were prepared (glucose contains 40% carbon by mass) and analyzed using the same digestion and titration procedure. A calibration curve ($FeSO_4$ volume vs OC equivalents) was used exclusively to assess method linearity and sensitivity.

Method Validation Parameters

Method validation was performed in accordance with ISO/IEC 17025:2017 requirements and ICH Q2(R1), supported by relevant ISO guidance (ISO 8466-1:2021; ISO 11843-2:2000; ISO 5725-2:2025). The following performance characteristics were evaluated:

- Repeatability and trueness: BIPEA certified reference soil analyzed in 10 replicate titrations (n = 10); mean OC compared with certified value to estimate bias.
- Linearity: glucose standards (0.1–3.0% OC); regression parameters (slope, intercept, R^2).
- LOD and LOQ**:** calculated from the calibration slope (S) and the standard deviation (SD) of replicate responses at the lowest concentration level.
- Precision**:** intra-day repeatability and inter-day intermediate precision at 0.1% and 3.0% OC, expressed as RSD (%).

- Accuracy: standard addition recovery test in BIPEA matrix at low and high OC levels; recovery (%) calculated using stoichiometric quantification (Eq. 1).

Results and discussion

The analytical performance of the Walkley–Black titrimetric method for SOC determination was evaluated through replicate analyses and a structured validation approach. Two complementary quantification strategies were applied: (i) classical stoichiometric calculation based on blank–sample titration difference (primary quantification approach for BIPEA trueness assessment and standard addition recovery testing), and (ii) calibration-based response evaluation using glucose standards and linear regression (used exclusively for linearity assessment and sensitivity estimation). In all calculations, the oxidation correction factor of 1.33 was applied.

BIPEA Reference Material – Accuracy Assessment

To evaluate repeatability and trueness of the Walkley–Black titrimetric method under the applied laboratory conditions, the BIPEA certified reference soil was analyzed in ten independent replicate titrations (n = 10). The $FeSO_4$ volume consumed for the BIPEA sample (Va) ranged from 14.0 to 14.5 mL, yielding a mean value of 14.26 mL (SD = 0.18 mL; RSD = 1.26%) (Table 1). Organic carbon was calculated for each replicate using the classical Walkley–Black stoichiometric equation based on the blank–sample titration difference (Eq. 1), applying the conventional correction factor of 1.33 to account for incomplete oxidation efficiency (~77%). The calculated OC values ranged from 1.70 to 1.80%, with a mean OC content of 1.748% (≈1.75%) (SD = 0.04%; RSD = 2.10%) (Table 1), confirming good repeatability of the method. When compared with the BIPEA certified value (1.97%; tolerance interval 1.72–2.14%), the experimentally obtained mean OC fell within the certified acceptance range, supporting satisfactory trueness for this reference soil matrix. The observed negative bias (−0.22%; −11.27%) is consistent with the known limitations of dichromate wet oxidation, including incomplete oxidation of resistant organic carbon fractions and potential matrix-related effects (Williams et al., 2023).

The observed negative bias reflects the known limitation of the Walkley–Black method related to incomplete oxidation of resistant organic carbon fractions. Although the conventional correction factor (1.33) compensates for average oxidation efficiency, slight underestimation of SOC

compared to certified reference values can still occur depending on organic matter composition.

Table 1. BIPEA certified reference soil results: precision (repeatability) and trueness (n = 10)

Parameter	Result
Mean $FeSO_4$ volumefor BIPEA, Va (mL) ± SD	14.26 ± 0.18
RSD of Va* (%)	1.26
Mean OC** in BIPEA (%) ± SD	1.75 ± 0.04
RSD of OC (%)	2.10
Certified OC BIPEA (%)	1.97
Bias, OC (Measured – Certified) (%)	-0.22
Relative Bias (%)	-11.27

*Va is the $FeSO_4$ volume consumed during titration of the BIPEA sample. **OC (%) was calculated using (Eq. 1) based on blank–sample titration difference, applying the oxidation correction factor (1.33).

Linearity, Calibration Curve, LOD and LOQ

Linearity was assessed using glucose-derived OC standards (0.1–3.0% OC equivalents) titrated under identical conditions as soil material. A calibration curve was constructed by plotting $FeSO_4$ titration volume (mL) versus OC equivalents (%) and evaluated by linear regression according to ISO 8466-1:2021 (Figure 1). The method showed excellent linearity across the investigated working range (R^2 = 0.999), indicating a proportional titration response to oxidizable carbon concentration. Importantly, the calibration curve was used exclusively as a validation tool to confirm response linearity and sensitivity; SOC quantification in BIPEA and standard addition experiments was performed using the classical stoichiometric blank–sample approach to ensure direct comparability with certified reference values. The limits of detection (LOD) and quantification (LOQ) were estimated at the lowest calibration level (0.1% OC) using the expressions LOD = 3 × SD / S and LOQ = 10 × SD / S, where SD represents the standard deviation of replicate titration responses and S is the slope of the calibration curve, in accordance with the principles of ISO 11843-2:2000 for detection capability evaluation. The main regression and sensitivity parameters are summarized in (Table 2).

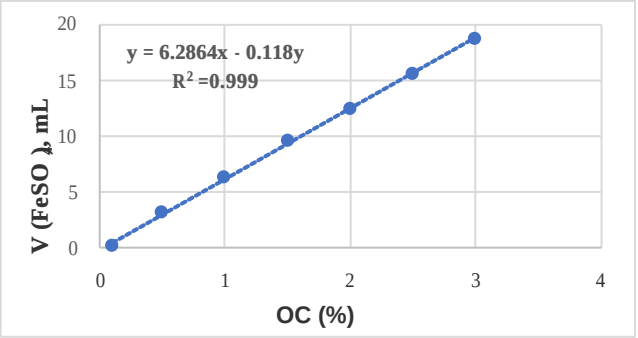

Figure 1. Calibration curve for glucose OC standards (0.1–3.0% OC) based on $FeSO_4$ titration volume.

Table 2. Linear regression data and sensitivity parameters

Parameter	Value
Concentration range (working)	0.1% – 3.0% OC
Slope (sensitivity)	6.2864
Intercept	−0.118
Coefficient of determination (R^2)	0.999
Limit of detection (LOD)	0.01% OC
Limit of quantification (LOQ)	0.04% OC

Method Precision: Intra-day Repeatability and Inter-day Intermediate Precision

Method precision was evaluated using relative standard deviation (RSD, %) at two concentration levels: low (0.1% OC) and high (3.0% OC). Precision was assessed as intra-day repeatability (three replicates within a single day) and inter-day intermediate precision (three replicates per day over three consecutive days; n = 9). The results are presented in (Table 3).

Precision was expressed as RSD (%) and evaluated according to ISO 5725-2:2025 and ICH Q2(R1). At 0.1% OC, intra-day and inter-day RSD values were 4.6% and 4.3%, respectively, indicating reliable method performance at low analyte concentrations where variability is typically higher. At 3.0% OC, very low RSD values (0.3% intra-day; 0.2% inter-day) were obtained, confirming excellent precision across the working range and strong endpoint consistency of the titration procedure.

Table 3. Inter-day and intra-day precision

Standard OC (%)	Intra-day RSD (%) (n = 3)	Inter-day RSD (%) (n = 9)
0.1 (Low-level concentration)	4.6	4.3
3.0 (High-level concentration)	0.3	0.2

Method Accuracy: Standard Addition Recovery Test

Accuracy was further evaluated by standard addition to the BIPEA certified reference soil at the lowest and highest concentration levels of the working range. In this recovery experiment, OC quantification was performed by the classical Walkley–Black stoichiometric approach (Eq. 1), applying the oxidation correction factor (1.33). This ensured direct assessment of matrix effects in a real soil matrix and avoided potential bias introduced by external calibration. Recovery (%) was calculated according to (Eq. 2).

$$Recovery\,(\%) = \frac{(Found\ OC - Initial\ OC)}{Added\ OC} \cdot 100\% \quad (2)$$

The obtained recoveries were 100% at the low-level addition and 99.3% at the high-level addition (Table 4), confirming excellent accuracy and negligible matrix interferences. Both values fall within the commonly accepted range (95–105%) for analytical method validation, supporting suitability of the method for routine SOC determination.

Table 4. Standard addition recovery results for BIPEA (n = 3)

Spike level	Initial OC (%)	Added OC (%)	Found OC (%)	Recovery (%)
Low level*	1.75	0.1	1.85	100.0
High level**	1.75	3.0	4.73	99.3

*BIPEA sample spiked with the low-level OC standard (0.1% OC); ** BIPEA sample spiked with the high-level OC standard (3.0% OC)

The standard addition results (Table 4) confirm that the method provides an accurate analytical response within the soil matrix and is not significantly affected by matrix interferences. In contrast, the negative bias observed for the BIPEA reference soil (Table 1) reflects the intrinsic limitation of the Walkley–Black method related to incomplete oxidation of resistant native organic carbon fractions rather than matrix effects.

Conclusion

The classical Walkley–Black titrimetric method was successfully validated for routine determination of soil organic carbon (SOC) under the applied laboratory conditions. The method demonstrated excellent linearity within the working range of 0.1–3.0% OC (R^2 = 0.999), confirming proportional analytical response. Repeatability testing using BIPEA certified reference soil (n = 10) showed good precision, with low variability in both titration volume (RSD = 1.29%) and calculated OC content (RSD = 2.10%). The obtained mean OC value (1.75%) fell within the BIPEA certified tolerance interval, indicating acceptable trueness despite a minor negative bias consistent with partial oxidation effects. Sensitivity was adequate (LOD = 0.01% OC; LOQ = 0.04% OC), and accuracy evaluation by standard addition confirmed minimal matrix influence (recovery 99.3–100%). Overall, the Walkley–Black titrimetric method proved to be a rapid, reliable, and cost-effective approach suitable for routine SOC determination in agricultural soil laboratories.

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