21 Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
9. – 11. rujna 2026. • Vukovar • Hrvatska

A novel spectrophotometric method for nitrate determination: assessment using green and white analytical chemistry metrics

Anita Martinović Bevanda[1], Marina Šuman[1], Ante Prkić[2], Anita Ivanković[3], Stanislava Talić[1]

[1]University of Mostar, Faculty of Science and Education, Matice hrvatske bb, 88 000 Mostar, Bosnia and Herzegovina
[2]University of Split, Faculty of Chemistry and Technology, Ruđera Boškovića 35, 21 000 Split, Croatia
[3]University of Mostar, Faculty of Agronomy and Food Technology, Biskupa Čule bb, 88 000 Mostar, Bosnia and Herzegovina

1 INTRODUCTION

Excessive nitrogen compounds nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) from agriculture and wastewater drive aquatic eutrophication, causing algal blooms and oxygen depletion that threaten ecosystems and human health.
This work presents a novel, eco-friendly spectrophotometric method for nitrate determination in water.

➤ **Analytical Mechanism:** Zn powder reduces nitrate (NO_3^-) to nitrite (NO_2^-) followed by the Griess reaction to form a colored complex for spectrophotometric measurement

➤ **Key Innovations:** Replaced toxic cadmium with safer zinc while optimizing reaction conditions to minimize reagent consumption, reduction time, and sample volume

➤ **Sustainability Assessment:** Evaluated and compared against three established methods using green chemistry metrics

➤ **Green Chemistry**
The design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances.

➤**Two evaluation frameworks**
- GAC focuses on eco-friendliness
- WAC integrates environmental safety, analytical performance, and practical feasibility into a single balanced model

2 KEY FINDINGS ON NOVEL METHOD FOR NITRATE DETERMINATION

- **Table 1.** Overview of the optimized parameters

PARAMETER	TESTED RANGE	OPTIMUM
pH	3.0–9.0	8.5
Zn slurry concentration	0.012–0.1 g mL^{-1}	0.085 g mL^{-1}
Reduction time	1–10 min	3 min
Reaction time with GR*	1–30 min	9 min
GR* volume	20–100 µL	25 µL

* GR – Griess reagent

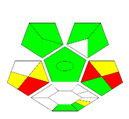
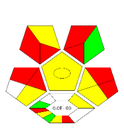
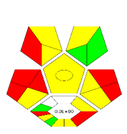
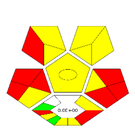
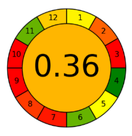
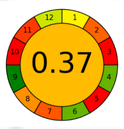
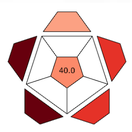
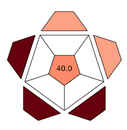
- **Table 2.** Analytical parameters of the optimized method for nitrate determination

(LDR), mol L^{-1}	Calibration equation, (c×10^{-5} mol L^{-1})	LOD, mol L^{-1}	LOQ, mol L^{-1}	R^2	RSD % $c(NO_3^-) = 1.0×10^{-5}$; n = 10
$3.0 × 10^{-7}$ – $1.0 × 10^{-4}$	A = 0.198c+0.0636	$1.8 × 10^{-7}$	$5.5 × 10^{-7}$	0.998	1.59 %

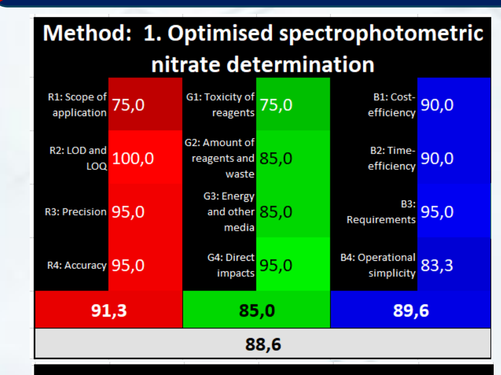
3 COMPARATION OF ANALYTICAL METHODS FOR NITRATE DETERMINATION

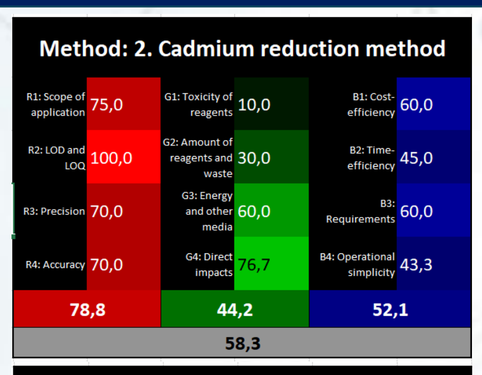
METHOD	ANALYTICAL PRINCIPLE	KEY REAGENTS USED
METHOD 1 Novel optimised spectrophotometric nitrate determination	Zinc-mediated reduction of NO_3^- to NO_2^-, followed by the Griess reaction (diazotization/coupling) to form a pink azo dye measured spectrophotometrically at λ = 540 nm.	• Ammonia buffer (pH 8.5) • Zinc slurry (0.085 g Zn/mL glycerol) • Griess reagent (Sulfanilamide, N-(1-naphthyl)ethylenediamine dihydrochloride, conc. HCl).
METHOD 2 Cadmium reduction method	Reduction of NO_3^- to NO_2^- using a copperized cadmium (Cu-Cd) glass column, followed by diazotization with sulfanilamide, coupling with NED, andspectrophotometric measurement	• Copper-cadmium granules • Ammonium chloride-EDTA solution (NH_4Cl-EDTA) • Color reagent (Sulfanilamide and N-(1-naphthyl) ethylenedi amine dihydrochloride - NED) • Hydrochloric acid (HCl) • Copper sulfate solution ($CuSO_4$)
METHOD 3 Spectrophotometric determination of nitrate with a single reagent	Acidic reduction of NO_3^- to NO_2^- using vanadium(III) chloride (VCl_3), followed by a reaction with Griess reagents (sulfanilamide and NEDD) and spectrophotometric quantification.	• Vanadium(III) chloride (VCl_3) • Hydrochloric acid (HCl) • Sulfanilamide • N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD)
METHOD 4 ISO 7890-3:1988 Determination of nitrate Part 3: Spectrometric method using sulfosalicylic acid	Reaction of nitrate with sodium salicylate in concentrated sulfuric acid to form a nitrosalicylate compound, which turns yellow upon alkalization and is measured spectrophotometrically.	• Sodium salicylate ($C_7H_5NaO_3$) • Concentrated sulfuric acid • Alkaline solution (NaOH + $EDTANa_2$) • Sodium azide (NaN_3) • Glacial acetic acid (CH_3COOH)

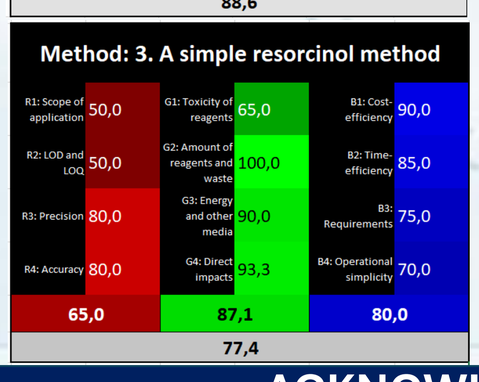
4 EVALUATION OF GREENNESS METRICS

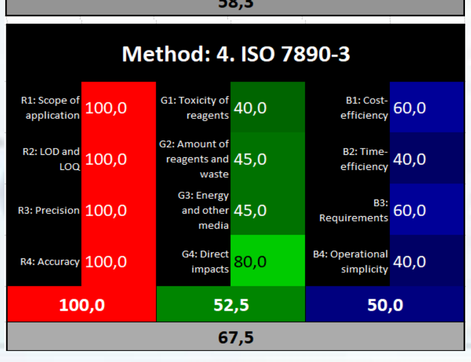
METRICS FOR GREENNESS	METHOD 1	METHOD 2	METHOD 3	METHOD 4
ComplexGAPI				
AGREE				
BAGI	70	60	67.5	57.5
RAPI	55	 40	 40	42.5

5 MULTIDIMENSIONAL WAC ANALYSIS (RGB MODEL)









6 CONCLUSION

🎯 **OPTIMAL ANALYTICAL PERFORMANCE AND SUSTAINABILITY:**
Method 1 - the top White Analytical Chemistry (WAC) score of 88.6. It offers an optimal balance by replacing toxic cadmium with a safer Zn/glycerol slurry, integrating high analytical performance (91.3), practical workflow (89.6), and environmental safety (85.0)

✅ **HIGHEST METHODOLOGICAL RELIABILITY AND ISO-VALIDATED QUALITY**
Method 1 - the highest RAPI score (55). offering superior analytical reliability and precise performance metrics, despite its lower environmental score.

🌱 **HIGHEST ECO-EFFICIENCY AND USE OF SMALL SAMPLES:**
Method 1 and Method 3 - the highest eco-efficiency (AGREE: 0.63/0.61) and practical applicability (BAGI: 70/67.5). Its micro-volume VCl_3 reduction with a microplate reader makes it the ideal green approach for rapid multi-sample analysis.

☣️ **LEAST SUSTAINABLE AND HIGH-HAZARD:**
Method 2 - the lowest eco-sustainability indicators (AGREE: 0.36, WAC: 44.2) due to the heavy environmental and health risks associated with the copperized cadmium column, hazardous buffer waste and excessive use of chemical reagents.

7 ACKNOWLEDGEMENTS

This work is supported by Federal Ministry of Education and Science, Bosnia and Herzegovina, Grant 05-35-3507-1/25, Application of ecological metrics in the validation of analytical methods and the quantitative assessment of their "greenness", (Primjena eko-metrike pri validaciji analitičkih metoda i kvantitativnoj procjeni njihove „zelenosti")