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ARTICLE

Determination of Basic Nitrogen Content in Diesel Oil

A Miniaturized Method by Digital Image-based Colorimetry in a Portable Device

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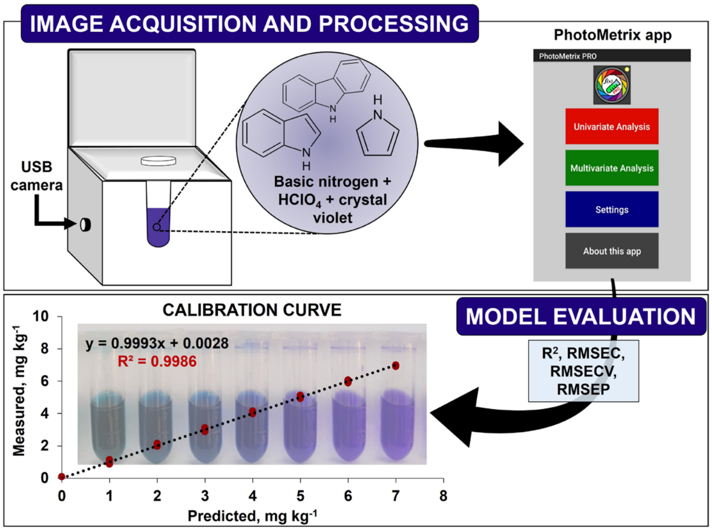

A miniaturized method using a portable device with digital image acquisition and PhotoMetrix PRO app data treatment was developed for the determination of basic nitrogen content in diesel oil. The method was based on the colorimetric titration described in the UOP 269-10 standard method. A homemade 3D-printed chamber with controlled light intensity equipped with an USB camera was used for image acquisition after an acid-base titration reaction, carried out in a miniaturized device. After mixing reagents and diesel oil, the images were obtained and converted into RGB (red, green, and blue) histograms, and a partial least squares (PLS) multivariate calibration model was constructed. Parameters of the regression model were evaluated, by the coefficient of determination (R^2), the root mean squared error of calibration (RMSEC), the root mean squared error of cross-validation (RMSECV), and the root mean squared error of prediction (RMSEP). Some conditions for the acid-base titration were optimized, such as the concentration of the indicator (68.0 to 272 μmol L^{-1}) and the titrating ($HClO_4$, 0.179 to 1.79 mmol L^{-1}), as well as the volume of diesel oil. With 60 μL of 2.54 mmol L^{-1} indicator solution, 20 μL of 20 mmol L^{-1} $HClO_4$ as titrating and using 50 to 1000 μL of diesel oil, optimal conditions were obtained for calibration (RMSEP of 0.377 mg kg^{-1}, RMSECV of 0.307 mg kg^{-1} with 4 factors). It is important to mention that no differences were observed ($p < 0.05$) when comparing reference values with the results by the proposed protocol. This proved to be advantageous in relation to the methods described in the UOP 269-10 standard since it was possible to reduce the consumption of reagents and waste generation, in agreement with green analytical chemistry. In addition, this alternative protocol combines simplicity and speed to obtain results with good accuracy, precision and suitable limit of quantification (1 mg kg^{-1}), using a miniaturized system.

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Keywords: diesel oil, basic nitrogen, PhotoMetrix PRO, miniaturized method

INTRODUCTION

Diesel oil is a crude oil fraction mainly used as a fuel in marine vessels, automobiles, civil construction, agricultural and locomotive machinery, in addition to power generating units. This fuel is obtained from crude oil distillation with some contaminants, mainly sulfur and nitrogen compounds.[1,2] Among the most common nitrogen compounds are quinolines, carbazoles, pyridines, indoles, pyrroles, acridine, and aniline.[1-3] Those with basic behavior (mainly pyridine and quinoline) can affect the catalysts used in the processing units, act as poisoners (at low temperatures) or inhibitors (at high temperatures), making the process difficult and increasing costs.[4,5] Furthermore, these basic compounds can react with acids present in crude oil, such as carboxylic acids and/or acids formed during the refining process, which can result in the formation of organic salts, leading to encrustation and clogging of equipment and pipes.[2,6] In general, nitrogen compounds can change color, odor, and stability of final products.[7] Nitrogen-neutral compounds (such as pyrroles, indoles, and carbazoles) can directly affect processes and equipment in crude oil processing units by forming gum/polymerization and can restrict the catalytic activity on hydrodesulfurization (HDS).[4,5,8] Besides that, nitrogen compounds are linked to environmental impacts, such as atmospheric pollution through the emission of particulate matter, polluting gases, and acid rain.[1-3]

To decrease the environmental impact due to the use of fossil fuels, regulatory agencies in several countries have been implementing programs to reduce emissions,[9] and standard methods are stablished to monitor contaminant levels in fuels.[10] In Brazil, the National Agency of Petroleum, Natural Gas and Biofuels (Agência Nacional de Petróleo, Gás Natural e Biocombustíveis, ANP) defines the specifications of diesel oil.[6] Although there is no legislation limiting the amount of total nitrogen in diesel oil (such as for sulfur), it is essential to know its concentration, as well as the types of compounds, mainly the basic fraction. In contrast to the set of options available for total sulfur determination and determination of sulfur compounds, nitrogen determination is less common, and just a few methods are available. According to standard methods, total nitrogen determination can be performed using total analyzers and acid digestion followed by titration.[11-14] Each standard describes a technique that suggests the type of sample that is covered, as well as suggest a nitrogen concentration range to assure accuracy. The most common choice for total nitrogen in diesel oil is ASTM D 4629 that consists in a controlled fuel combustion in a heated tube followed by chemiluminescence detection.[12]

When trying to understand the profile of nitrogen content (that means, differentiating basic and non-basic nitrogen) analytical methods are most devoted to the screening and quantification by separation techniques (mainly chromatography) hyphenated to element-specific detection or mass spectrometry.[15,16] In spite of their suitability and powerful detection capability, such techniques entail a higher cost to implement in laboratory routine, as well as can be carried out following relatively time-consuming protocols, from sample preparation to detection.[7,15,17] Thinking about having simple strategies for quality control laboratories, it is interesting to develop methodologies that are low cost and provide quick response, even if they do not allow the speciation of nitrogenous compounds, but simply provide the determination of the basic fraction.

To obtain this information, the UOP 269-10 standard method describes two protocols for determining the basic nitrogen content in fuels. The application of such a standard does not require any sample preparation step and can be easily implemented in routine laboratories. The described methods are based on titrimetric analysis with potentiometric or colorimetric detection to identify the end of the titration.[18] However, one of the main disadvantages of using the methods described in this standard is the consumption of a large amount of sample (about 100 mL) and reagents (mainly acetic acid, about 200 mL per run), which is the opposite of what is recommended in the principles of green chemistry.[19] Considering this aspect, the development of alternative methods is an important issue.

Colorimetry is based on measuring color to describe it numerically. Color perception can be influenced by some parameters, such as the nature of lighting, optical properties (reflection and transmission), and the response of the human eye. In a simple way, colorimetry can be based on the separation of RGB color components (R-red, G-green, B-blue), working in an analogous way to the system of human vision.[20,21]

Obtaining digital images for analytical purposes has gained increasing interest, being a simple method, easily applicable, and providing a quick response.[22] In this sense, colorimetry can be easily combined with obtaining digital images by cell phones, photographic cameras, endoscopes, and scanners, among others. This combination is possible since the digital image has pixels containing the primary colors: red, green, and blue. In each pixel, there are different values (0 to 255) of each color, generating several combinations and, consequently, colors. Helfer et al.[23] proposed a free smartphone application called PhotoMetrix, capable of obtaining digital images, treating them, proposing chemometric models, and evaluating their respective figures of merit, allowing several applications. It is available on digital platforms for Android or Windows phone operating systems and has two versions for capturing images: the PRO version (smartphone camera) and the UVC version (external camera, e.g. endoscope).[23]

After obtaining the images, the application converts the RGB channels into their respective histograms, making conventional univariate analysis possible, as well as multivariate analysis using chemometric models. In addition, the simplicity of using the application, the speed of obtaining and processing data, the intuitive interface, and obtaining immediate results make this approach advantageous for some developments. One of the main advantages of this application is the possibility of calibration in different color scales, not limiting the applications to colorimetric methods that have only a single color scale.[23]

Several studies have demonstrated the combination of colorimetry and digital images for analytical purposes, including some protocols for fuels. The use of digital imaging has proven to be efficient for quickly determining the amount of biodiesel present in diesel.[24] Other studies have demonstrated the application of this approach for the determination of salt in crude oil,[25] and calcium and magnesium in biodiesel.[26]

Based on the aforementioned aspects, as well as the need to characterize the basic nitrogen content in diesel oil without sample pre-treatment, combined with the disadvantages associated with the official standard (slowness, use of large amounts of sample and reagents), it is of paramount importance to develop a method that can be suitable and advantageous from the analytical point of view. Ideally this method should be simple, robust, low cost, and, preferably, miniaturized. Thus, the objective of this work, was the development of a miniaturized protocol based on colorimetry with digital images in a portable device for determining the basic nitrogen content in diesel oil without sample preparation, with minimum consumption of reagents and simple instrumentation.

MATERIALS AND METHODS

Instrumentation

Colorimetric analysis with digital image was performed using a portable device equipped with a 7 mm USB camera (intelligent endoscope model, B-Max, China) with a resolution of 640 x 480 pixels and a 6 W light emitting diode (LED). The device was designed (Solidworks 3D Premium 2008, USA) and printed by fused deposition using polylactic acid (PLA) in a 3D printer (model Core A3 V2, GTMaX 3D, Brazil).

Images were acquired using an USB camera connected to a notebook (Ideapad S145 model, Lenovo, Intel Core i7-8565U, Brazil). The PhotoMetrix PRO application (free for download on Android, iOS, and Windows systems) was used to obtain images and process data. To use the application on the notebook, it was necessary to install an emulator (BlueStacks App Player, Bluestacks System Incorporated, USA), also available free of charge. The colorimetric reaction was performed inside a 2 mL round bottom flask (Cralplast, Brazil), which was inserted inside the portable device for image acquisition. Figure 1 shows the system and a typical analysis run (in detail).

Reference values for basic nitrogen content were obtained following the UOP 269-10 standard method, by potentiometric titration. An automatic titrator (model Titrando 836, Metrohm, Switzerland) with magnetic stirring and a combined pH glass electrode for non-aqueous medium (model 6.0262.100, Metrohm, Switzerland) were used.

Total nitrogen content in diesel oil was determined using a total nitrogen and sulfur analyzer (Antek Instruments, model 9000 series NS, USA) following the ASTM D 4629-04 standard with direct sample injection.[12]

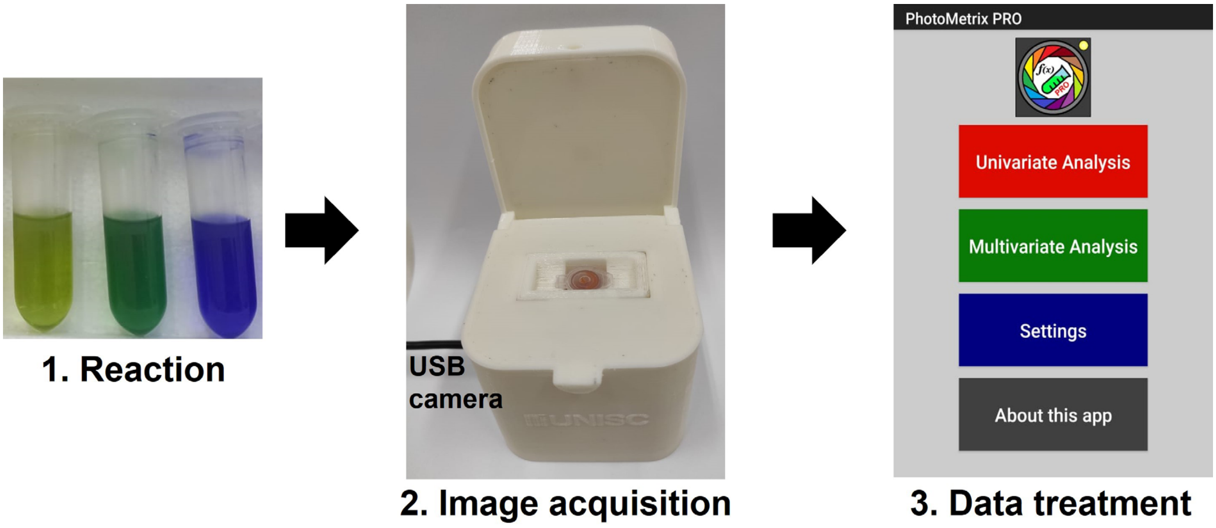


Figure 1. Homemade portable device used for the acquisition of images: the first step corresponds to reagents and sample mixing for the colorimetric reaction (1), followed by the second step that is the image acquisition by adding the flask into the homemade device (2), and the third step corresponds to the data acquisition and processing (3).

Reagents and samples

The determination of basic nitrogen was performed using a 10 mmol L^{-1} $HClO_4$ solution (colorimetric method) or 2 mmol L^{-1} $HClO_4$ solution (potentiometric method), prepared from $HClO_4$ P.A. (70%, Merck, Brazil) in glacial acetic acid (Vetec, Brazil). The titrant was standardized with tris(hydroxymethyl) aminomethane (THAM, Merck, Brazil) solubilized in glacial acetic acid. The crystal violet indicator used for all experiments (2.54 mmol L^{-1}) was prepared in glacial acetic acid according to the UOP 269-10 standard by dissolving 0.1 g of the reagent in 100 mL of solvent. The calibration curve (0 to 10 mg kg^{-1} of N) was made using pyridine P.A (Merck, Brazil) solubilized in glacial acetic acid (Vetec, Brazil) for total nitrogen determination. These same reagents were used for the development of the colorimetric method. For this, the calibration curve was prepared by sequential dilution of a 70 mg kg^{-1} stock nitrogen solution prepared from pyridine P.A (Merck, Brazil), in glacial acetic acid (Vetec, Brazil), with specific volumes of indicator and $HClO_4$.The amount of nitrogen standard, indicator, $HClO_4$, and acetic acid changed according to the parameter under investigation for each set of experiments, but the total volume inside the flask was always maintained at 1120 µL. The exact values for each reagent are listed in Table S1 (Supplementary Material).

The samples selected for the development of this work were identified as DO1 to DO10. They were acquired at local gas stations (DO1 to DO5 and DO10), as well as were available from previous technical agreements where they were used for other studies (DO6 to DO9). The sample DO1 to DO5 were labeled as “S10 type” (up to 10 ppm of S) at the gas station and the sample DO10 was labeled as “S500 type”. Diesel oil DO9 was used to optimize the method conditions since it contains a higher concentration of nitrogen and a greater amount was available.

Reference procedure: determination of basic nitrogen content

The basic nitrogen content was determined according to the UOP 269-10 standard[18] by potentiometric titration. About 1 g of diesel oil was placed in a flask, solubilized in 5 mL of toluene and 30 mL of acetic acid. Then, the titration was performed with a previously standardized 2 mmol L^{-1} $HClO_4$ solution in acetic acid medium. The end of the reaction was monitored with a pH electrode for non-aqueous medium. For comparison, basic nitrogen content was also determined using the colorimetric method described in the same standard. For this, about 25 g of diesel oil was solubilized in 100 mL of acetic acid and titrated with a previously standardized 10 mmol L^{-1} $HClO_4$ solution in acetic acid medium. The end of the reaction was monitored by the color transition from purple to dark blue.

Method development optimization: miniaturized colorimetric method in a portable device

The development of a protocol for determining basic nitrogen in diesel oil was performed using the homemade system with an USB camera to obtain the images and PhotoMetrix PRO for processing and treatment. Multivariate calibration mode by partial least squares (PLS) regression was selected in the PhotoMetrix PRO app, using the values obtained by the intensity variations of the RGB channels. Experiments for optimization were carried out using standard solutions of basic nitrogen (pyridine, 0 to 10 mg kg^{-1} N), with the addition of $HClO_4$ (0.179 to 1.79 mmol L^{-1}) and crystal violet indicator (68 to 272 µmol L^{-1}). The volumes of the solutions were evaluated by keeping the volume inside the flask constant at 1120 µL with acetic acid. For the calibration curve (0 to 10 mg kg^{-1} N), a specific amount of standard solution was mixed with $HClO_4$, indicator, and acetic acid, directly into a microtube (2 mL, conic flask). The same procedure was carried out for sample analyses, but the sample mass was changed depending on the basic nitrogen concentration. In both situations, the microtube was placed inside the homemade chamber and the images were acquired. The images were obtained right after the preparation of the standards, with a region of interest of 64 x 64, lighting at 80 lx (maximum possible for the system), and the camera at a distance of 2.19 mm from the flask. All PLS models were evaluated according to the coefficient of determination (R^2), the slope and the interception values, the root mean squared error of calibration (RMSEC), the root mean squared error of cross-validation (RMSECV), and the root mean squared error of prediction (RMSEP).

After the univariate optimization, a rotational central composite design (RCCD) of 8 experiments, including six axial points and four repetitions at the central point was carried out. Three independent variables were evaluated, which were defined as indicator volume, $HClO_4$ volume, and the concentration of $HClO_4$. This RCCD was carried out using DO9 sample, and the coded values and real values, are shown in Table I. The results were evaluated using StatSoft Statistica Enterprise software, version 10.0, 2010.

Table I. Rotational central composite design variables and their real/codified values for the optimization of the colorimetric method for basic nitrogen compounds in diesel oil

Coded values	Independent variables		
	Indicator (µL)	$HClO_4$ (µL)	$HClO_4$ (mmol L^{-1})
-1.68	1.20	3.20	3.20
-1	25	10	10.0
0	60	20	20.0
+1	95	30	30.0
+1.68	118.8	36.8	36.8

RESULTS AND DISCUSSION

Reference procedure: determination of basic nitrogen content

Initially, all diesel oil samples were determined in terms of basic nitrogen content, following the potentiometric and colorimetric titration protocol, both described in the UOP 269-10 standard method. The results by the potentiometric method were considered reference values for the optimization of the alternative method with the miniaturized system and are presented in Table II. In general, the diesel oil samples presented low concentrations of basic nitrogen and total nitrogen content from 28.7 to 154 mg kg^{-1}. Based on the results, the DO9 sample was selected for the development of the miniaturized method. In addition, the sample (DO2) was also used during the experiment to check the method accuracy for samples with a low concentration of basic nitrogen.

Table II. Basic nitrogen content in diesel oil, following the UOP 269-10 standard method and total nitrogen content by ASTM D 4629

Sample	Density[a] g cm^{3}	Basic nitrogen content[b] mg kg^{-1}		Total nitrogen content[b] mg kg^{-1}
		Potentiometric method	Colorimetric method	
DO1	0.8427	< 5	nd	40.2 ± 0.2
DO2	0.8417	< 5	nd	28.7 ± 0.8
DO3	0.8501	27.0 ± 3.0	20.4 ± 4.5	116 ± 10
DO5	0.8298	< 5	< 0.5	< 10
DO6	0.8430	< 5	nd	< 10
DO9	0.8664	66.3 ± 6.0	62.2 ± 1.9	154 ± 6
DO10	nd	20.9 ± 2.0	23.9 ± 2.4	85.1 ± 1.8

[a] Determination performed at 20 °C. [b] Values expressed as average ± standard deviation. nd: not determined (insufficient amount of sample).

Method development optimization: miniaturized colorimetric method in a portable device

The standard method for determining basic nitrogen in fuels describes two titration procedures: one potentiometric and the other colorimetric. For both, the determination can be performed directly on diesel oil, without the need of using a sample pre-treatment step. Considering that both standards recommend using high volumes of reagents (mainly acetic acid and toluene), a miniaturized procedure was optimized to reduce waste generation.

Since colorimetric titration is more viable for miniaturization, as well as a reduced cost in relation to the potentiometric one, an investigation was started to improve this methodology, using a portable system and digital images. Some conditions of the method, such as the camera focal length, the light intensity, and the region of interest, were based on the literature.[25] For the miniaturization of the colorimetric titration, the evaluations were made in univariate analysis and the following parameters were investigated: the concentration of indicator (by changing the added volume in the flask), and the concentration of $HClO_4$. It is important to mention that the colors observed in the experiments for basic nitrogen could vary among yellow, green, blue, or purple, with different intensities for each one. Considering this, all the parameters were evaluated by making a calibration curve containing pyridine (in an acetic acid medium) as a model compound for basic nitrogen, at concentrations of 0, 0.1, 0.5, 1, 5, and 10 mg kg^{-1} N. All calibration curves were evaluated according to the number of samples and factors, the coefficient of determination (R^2), the RMSEC, the RMSECV, and the RMSEP of the multivariate PLS model, selected directly in the PhotoMetrix PRO. It should be mentioned that for all procedures, the proportion of pyridine standard:acetic acid volume was maintained at 1:1, as well as the total volume inside the flasks at 1120 μL. Additionally, the number of standard solutions used to obtain the PLS model and to calculate the RMSEP models was kept constant for all experiments. For the calibration, four replicates of each standard (ranging from 0 to 10 mg kg^{-1} of basic nitrogen) were used, totalizing 24 solution flasks. To calculate the RMSEP models, 20 standard solutions (at least), and two diesel oil samples (containing different concentrations of basic nitrogen) were used, and each one was analyzed four times.

Finally, it is important to mention that according to the UOP 269-10 standard method, a color change occurs depending on the reaction between basic nitrogen, perchloric acid, and crystal violet indicator. The color starts yellow at the beginning of the titration (meaning high basic nitrogen content) and changes to purple passing through green and blue. Thus, when the purple color is observed, there is an excess of perchloric acid, and no further color change can be observed. This change of color scale is also considered to evaluate the calibration curve and help to choose the best PLS model.

Indicator concentration

Considering that the indicator volume will affect the intensity of color formation, the PLS model response was evaluated for 30, 60, 90, or 120 µL, which corresponds to 68.0, 136, 204, and 272 µmol L^{-1}, respectively. For this experiment, a calibration curve (0 to 10 mg kg^{-1} of basic nitrogen) was evaluated for all indicator volumes, and the best condition was chosen according to the parameters obtained from the calibration curve and RMSEP values. Some conditions were kept constant, being the total volume in the tube as 1120 µL and with 60 µL of 20 mmol L^{-1} $HClO_4$. The results obtained are shown in Table III.

Table III. Results obtained by PLS model after colorimetric reaction and digital image acquisition for 0 to 10 mg kg^{-1} basic N by changing the indicator concentration (number of factors = 3)

Indicator concentration µmol L^{-1}	Slope	Intercept	R^2	RMSEC mg kg^{-1}	RMSECV mg kg^{-1}	RMSEP mg kg^{-1}
68.0	0.990	0.0249	0.990	0.375	0.960	1.88
136	0.996	0.0120	0.996	0.237	0.687	1.57
204	0.990	0.0283	0.990	0.360	1.15	4.20
272	0.996	0.0120	0.968	0.659	1.33	1.48

The best model presented a higher R^2, and the lowest error of calibration, cross-validation, and prediction (RMSEC, RMSECV, and RMSEP, respectively). Based on that, the best results were obtained using 136 µmol L^{-1} of indicator (60 µL), with better R^2 value and lower RMSEC and RMSECV values. Besides the RMSEP value being similar to the best condition, the use of a larger volume of the indicator (272 µmol L^{-1} or 120 µL of 2.54 mmol L^{-1}) was not feasible for this method, since it resulted in a quite intense coloring. Furthermore, it was the worst R^2 and resulted in the highest RMSEC and RMSECV values, probably as a consequence of that. As a low-resolution camera was used to obtain the images, the colors that were very intense ended up resulting in non-significant intensity variations at the RGB channels (Figure 2). This impaired adequate distinction between the standards from the calibration curve. In this sense, 60 µL of 2.54 mmol L^{-1} indicator solution (136 µmol L^{-1}) was chosen for the next experiments.

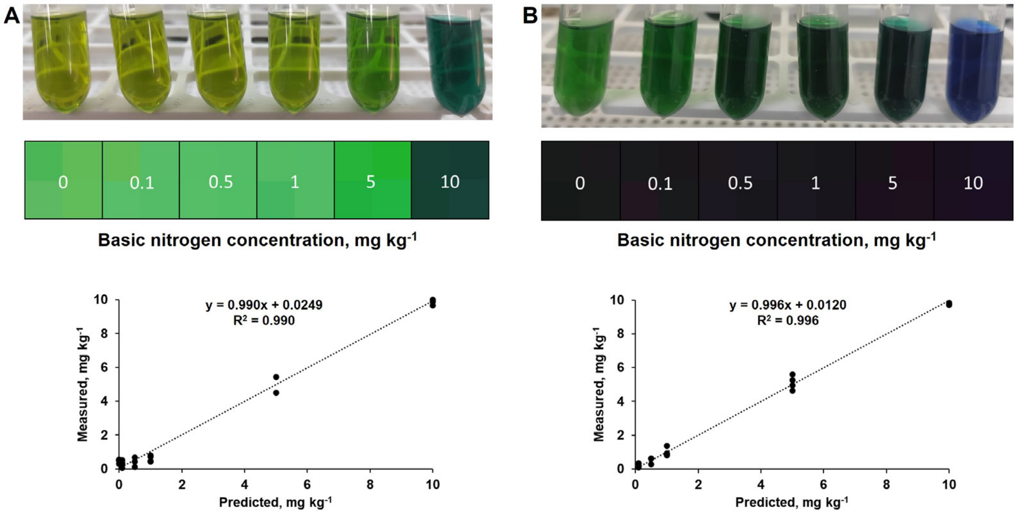


Figure 2. Calibration curve for basic nitrogen in the miniaturized system using (A) 68 µmol L^{-1} and (B) 272 µmol L^{-1} of the indicator using RGB channels and PLS model. Conditions: 60 µL of 20 mmol L^{-1} $HClO_4$ in acetic acid medium, n = 24 (four replicates per standard).

Evaluation of $HClO_4$ concentration

The concentration of $HClO_4$ is an important variable in the method since the acid must be at sufficient amount to neutralize the basic nitrogen compounds present in the medium, as well as to result in a suitable color to be identified in the images. Then, the concentration of the titrating $HClO_4$ was evaluated ranging from 0.179 to 1.79 mmol L^{-1} by adding 20 to 90 µL of 10 to 100 mmol L^{-1} $HClO_4$. The indicator volume (136 µmol L^{-1}) and the total volume inside the tube (1120 µL - made up with acetic acid) were kept constant.

As shown in Table IV, the smallest error of prediction (RMSEP of 0.752 mg kg^{-1}) was observed using 0.357 mmol L^{-1} (which corresponds to 20 µL of the 20 mmol L^{-1} $HClO_4$). It is possible to observe that the RMSECV had a slight variation for the different concentrations of $HClO_4$ evaluated. However, an opposite behavior was observed for the prediction error. The use of 1.79 mmol L^{-1} resulted in a prediction error about four times higher than using 0.357 mmol L^{-1} of $HClO_4$. Therefore, the best condition that resulted in lower errors was using 0.357 mmol L^{-1} of $HClO_4$ (equivalent to 20 µL of 20 mmol L^{-1} $HClO_4$).

Table IV. Results obtained by PLS model after colorimetric reaction and digital image acquisition for 0 to 10 mg kg^{-1} basic N as a function of $HClO_4$ concentration

$HClO_4$ mmol L^{-1}	Slope	Intercept	R^2	RMSEC mg kg^{-1}	RMSECV mg kg^{-1}	RMSEP mg kg^{-1}
0.179	0.995	0.014	0.995	0.265	0.778	2.61
0.357	0.975	0.077	0.974	0.595	0.960	0.752
0.714	0.991	0.031	0.991	0.362	0.882	1.41
0.893	0.999	0.002	0.999	0.098	0.746	1.49
1.07	0.992	0.027	0.992	0.344	1.22	1.77
1.34	0.999	0.005	0.999	0.145	0.772	2.25
1.61	0.980	0.069	0.980	0.572	3.03	2.98
1.79	0.998	0.006	0.998	0.166	0.902	2.98

Rotational central composite design experiments: variables influence

An experimental design was performed to evaluate the influence of variables in the formation of color and, consequently, in the RGB system. The influence of the indicator volume, and the concentration and the volume of $HClO_4$ are shown in Figure 3, in a standardized Pareto chart. During the experiments, the formation of purple color was observed, which indicates an acidic medium (an excess of the titrating $HClO_4$). According to the Figure 3, it is possible to observe that for the R (red) channel, almost all variables (linear and quadratic) were significant and most of them demonstrated that it is necessary to reduce the amount to perform the experiments (-8.78 and -8.58 values, respectively for the concentration and amount of $HClO_4$). About the G (green) channel, it was observed that the amount of indicator volume was extremely significant and that lower quantities are better (-7.35 value). In addition, the volume and concentration of $HClO_4$ were significant when related to the indicator volume, but when evaluated each one alone, no significant influence was observed. The Pareto chart for B (blue) channel (Figure 3) shows a significant influence combining the volume and concentration of $HClO_4$, whose ratio is most influenced when higher concentration is used, combined with low volume of $HClO_4$ or vice versa. Surface responses (Figure 4) present the influence of each variable.

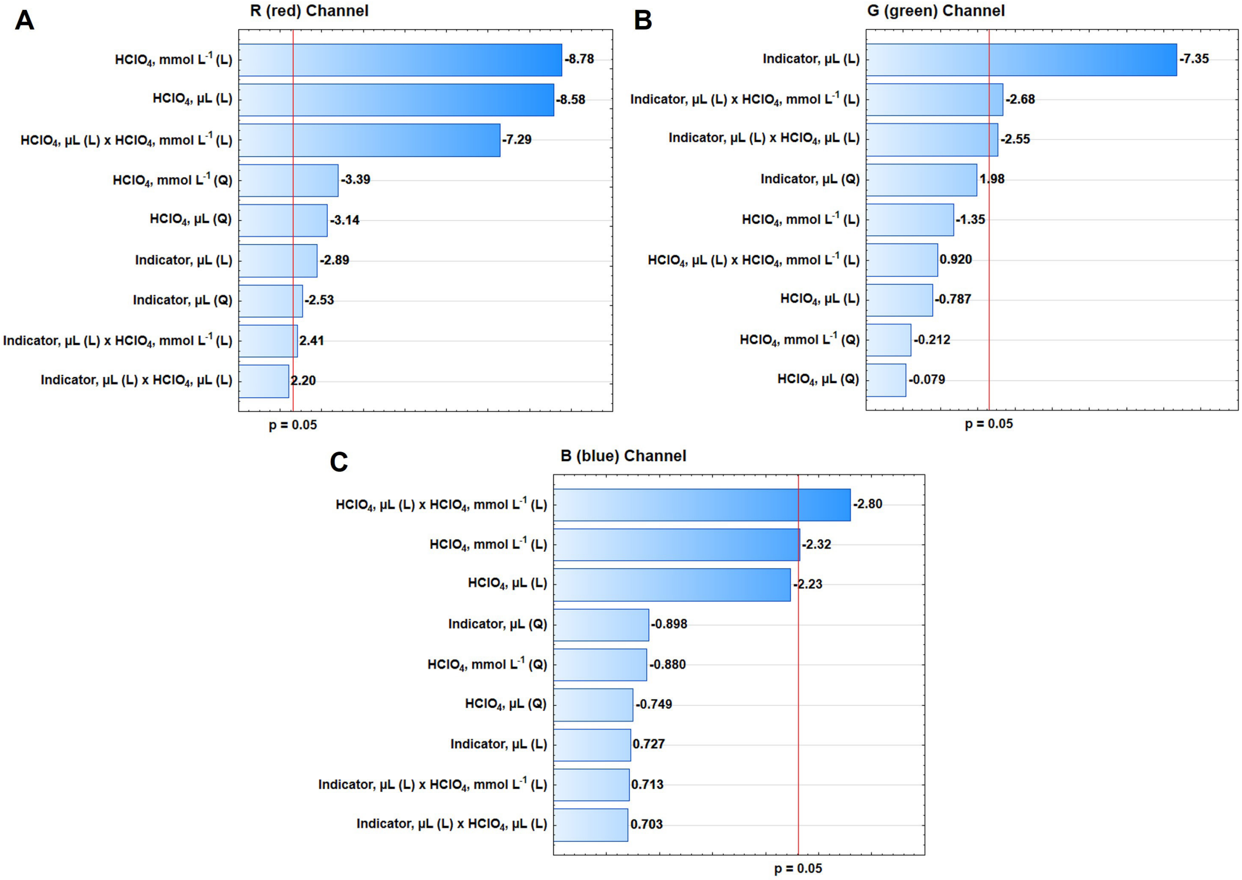
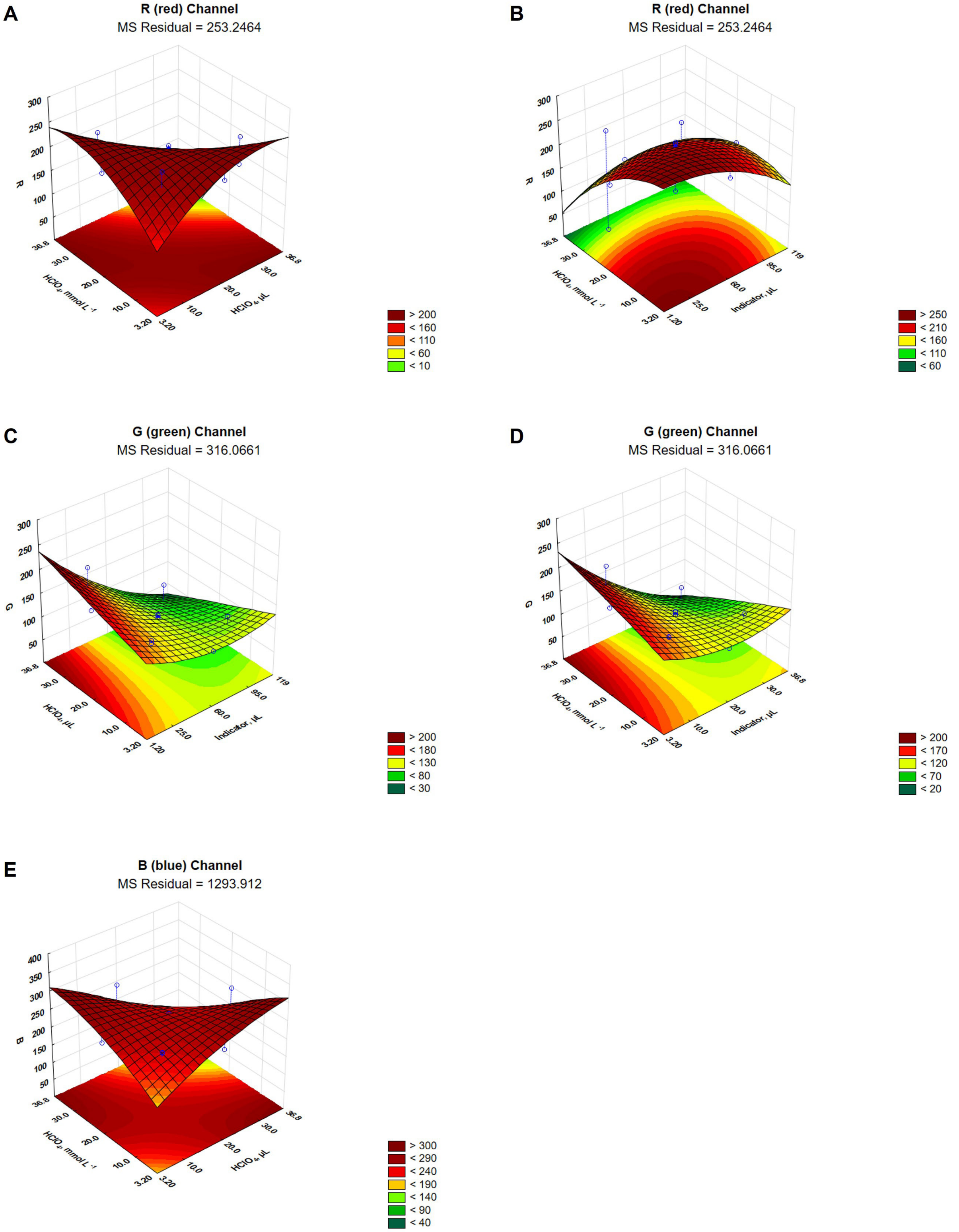


Figure 3. Standardized Pareto chart obtained for the results from RCCD experiments for (A) R (red) channel, (B) G (green) channel, and (C) B (blue) channel.



A R (red) Channel
MS Residual = 253.2464

B R (red) Channel
MS Residual = 253.2464

C G (green) Channel
MS Residual = 316.0661

D G (green) Channel
MS Residual = 316.0661

E B (blue) Channel
MS Residual = 1293.912

Figure 4. Surface responses for the results from RCCD experiments for: (*i*) R (red) channel comparing (A) $HClO_4$ volume *vs* concentration and (B) $HClO_4$ concentration *vs* indicator volume, (*ii*) G (green) channel for (C) $HClO_4$ volume *vs* indicator volume and (D) $HClO_4$ concentration *vs* indicator volume, and (*iii*) B (blue) channel for (E) $HClO_4$ volume *vs* concentration.

Analytical method parameters

After method development, analytical parameters were evaluated to assess the performance and feasibility of using the optimized method for diesel oil samples. Calibration was evaluated from 0.1 to 10 mg kg^{-1} of basic nitrogen, in order to cover samples over a wide range of concentrations. It was possible to obtain linearity and good response from 1 to 7 mg kg^{-1}, using 4 factors. Limits of detection (LOD) and LOQ were 0.880 mg kg^{-1} and 1 mg kg^{-1}. The results can be seen in Figure 5, showing R^2 greater than 0.99 and a prediction curve with R^2 of 0.98. From these experiments, good linearity was observed for both curves (calibration and prediction), and the optimized model was used to accurately quantify a set of samples (see in the next section). Furthermore, a RMSEP value of 0.377 mg kg^{-1} was obtained when analyzing a set of standard solutions as samples.

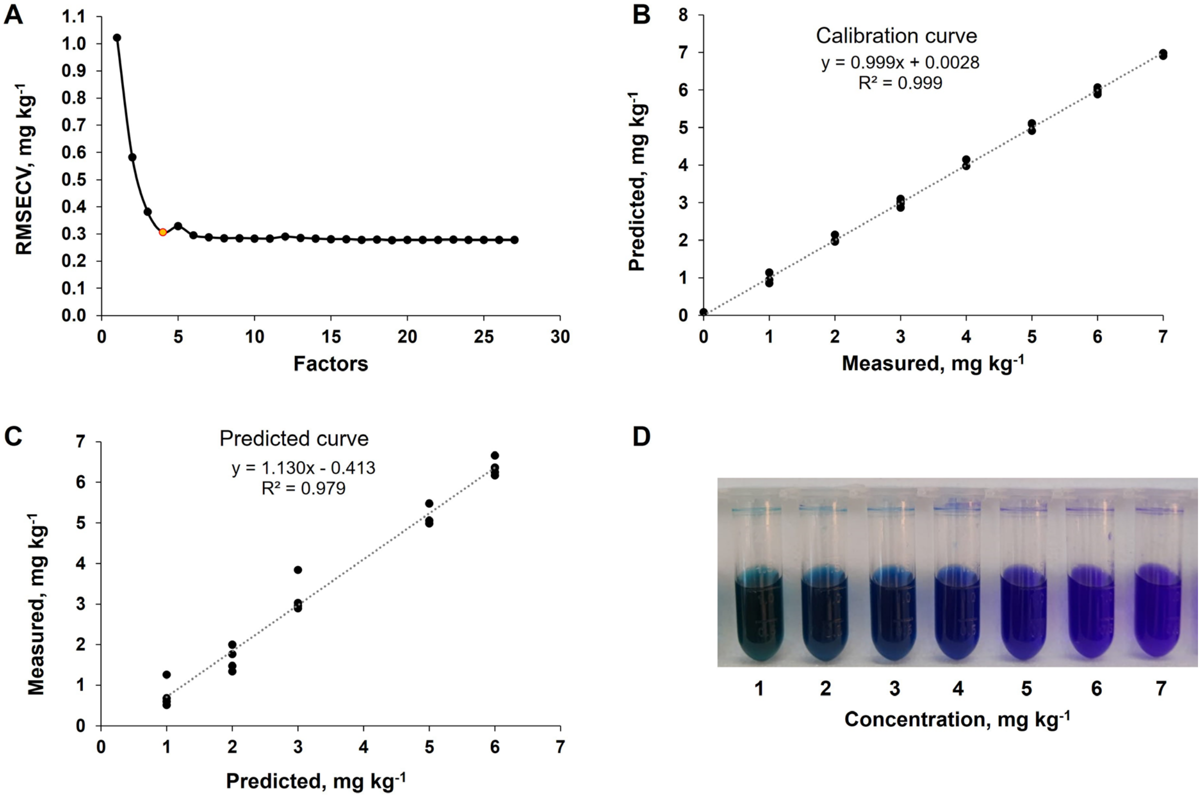


Figure 5. Parameters obtained for the calibration curve in the range of 1 to 7 mg kg^{-1}: (A) RMSECV values as a function of the number of factors, (B) calibration curve, (C) prediction curve, and (D) color variation for the set of solutions for the calibration curve, using the optimized conditions. Conditions: 136 µmol L^{-1} (60 µL) of indicator, 0.357 mmol L^{-1} $HClO_4$ (20 µL), total volume of 1120 µL.

Miniaturized colorimetric method for basic nitrogen determination: accuracy evaluation

After defining the suitable conditions, the method was applied to the diesel oil samples described in Table II. Initially, 520 µL of the DO9 sample was used, but the model did not respond well. It should be mentioned that diesel oil was not miscible in the medium and phase separation after addition of all reagents was observed. This behavior was the same for most of samples, probably as a consequence of additives. As the UOP 269-10 standard method suggests the use of a specific amount of diesel oil depending on the concentration of basic nitrogen, it was evaluated whether diesel oil volume would affect the model response. For this, volumes from 10 to 100 µL of the DO9 sample were evaluated and the results are presented in Table V.

Table V. Results for basic nitrogen content in diesel oil by colorimetry using a portable device and digital image with PhotoMetrix PRO for different sample amount (n = 4)

Diesel oil (µL)	Basic nitrogen concentration (mg kg^{-1})	Recovery (%)
10	137 ± 35	211 ± 54
20	40.2 ± 5.5	61.8 ± 8.4
40	61.6 ± 4.5	94.7 ± 6.9
60	66.2 ± 2.0	86.5 ± 3.1
80	74.7 ± 5.4	115 ± 4
100	61.7 ± 3.6	94.9 ± 5.7

DO9 = 66.3 ± 2.0 mg kg^{-1} of basic N.

By using 10 or 20 µL, it was observed that the model was not able to identify the basic nitrogen concentration with accuracy. From the use of 30 µL of sample, it was possible to obtain an agreement with the results from UOP 269-10 standard method (58.2 ± 3.0 mg kg^{-1} of basic N, 89.5 ± 4.6% of recovery). Thus, the model proved to be efficient to predict the concentration of basic nitrogen under optimal proportion. From this, the model's response to other samples was evaluated by choosing a suitable amount of sample and the results can be seen in Table VI.

Table VI. Results obtained for different samples of diesel oil applying the optimized miniaturized method and the UOP 269-10 standard method (n = 4). Conditions: 136 µmol L^{-1} of indicator, 0.357 mmol L^{-1} $HClO_4$, 1120 µL of total volume

Diesel oil	Basic nitrogen concentration (mg kg^{-1})		
	Miniaturized method	UOP 269-10 - Colorimetric method	Agreement (%)
DO1[a]	5.5 ± 0.4	nd	nc
DO2[a]	5.7 ± 0.2	nd	nc
DO3[b]	22.7 ± 2.4	20.4 ± 4.5	111
DO5[c]	< 1	< 0.5	nc
DO6[c]	< 1	nd	nc
DO9[d]	65.4 ± 4.4	62.2 ± 1.9	105
DO10[b]	20.7 ± 3.4	23.9 ± 2.4	115

The following volumes were used: [a]500 µL, [b]100 µL, [c]1000 µL, [d]50 µL.
nd: not determined (insufficient amount of sample). nc: not calculated.

From the results shown in Table VI, it was possible to see that the proposed method presented good results, since there was no significant difference (ANOVA, $p > 0.05$) when compared with those obtained by UOP 269-10 standard method using the colorimetric procedure. The amount of basic nitrogen fraction could be different depending on the origin of diesel/crude oil as demonstrated in previous reports (*e. g.* 214 µg g^{-1} and 580 µg g^{-1}).[28,29] Various studies have demonstrated the separation and identification of basic nitrogen from diesel oils with the use of sophisticated techniques, such as chromatography with mass spectrometry detector.[7,15] In spite of they are valuable tools, it is important to emphasize that the optimized method allows for such information in an easy-to-perform protocol. Furthermore, it was possible to determine low concentrations of basic nitrogen, making possible to use the optimized method as a replacement for the methods proposed in the UOP 269-10 method, avoiding the use of large amounts of sample and reagents.

As shown in Table VI, the amount of diesel oil changed according to the concentration range. The UOP 269-10 standard method describes the same approach, suggesting that the volume of diesel oil to be used varies among three different ranges of basic nitrogen concentration. Thus, a similar protocol was proposed for the miniaturized colorimetric method, as shown in Table VII.

Table VII. Guide for choosing the appropriate volume of diesel oil for the neutralization reaction described in the UOP 269-10 standard method and for the proposed method

UOP 269-10 standard method		**Miniaturized method**	
Estimated concentration of basic nitrogen (mg kg^{-1})	**Diesel oil volume (mL)**	**Estimated concentration of basic nitrogen (mg kg^{-1})**	**Diesel oil volume (mL)**
0.1 - 10	200	0.1 - 10	0.5
11 - 35	100	11 - 35	0.1
36 - 100	25	36 - 100	0.05

Green analytical aspects

After optimizing the miniaturized method for the determination of basic nitrogen, an assessment was made regarding the sustainability of the method, comparing it with the titration procedures described in the UOP 269-10 standard method, using the Analytical GREEnness-AGREE calculator.[19] This calculator is a tool capable of evaluating analytical methods considering the 12 principles of green analytical chemistry, which are transformed into a green, yellow, and red color scale.

Based on the criteria evaluated for each method, when comparing the colorimetric method described in the standard with the optimized miniaturized method, it was possible to verify that the sample mass was reduced by up to 250 times. Besides, it was possible to reduce approximately 100 times the volume of waste generated and a 5-fold increase in analytical frequency was possible. In Figure 6, a scale ranging from 0 to 1 is shown and refers to a general score of the method regarding sustainability. As close as possible to 1, the more sustainable the method is considered. It was possible to notice that the miniaturized method obtained a better score (0.62) while the methods described in the UOP 269-10 standard method obtained a score around 0.4, which classifies them as not very sustainable. Also, from the image, it is possible to verify that all three methods had low performance for principles 3 and 10 highlighted in red, since analyzes were not evaluated/used online mode and there was no use of reagents from renewable sources.

Using the miniaturized method, it was possible to comprise several of the principles of green analytical chemistry. It is possible to highlight principles 2, 5, 7, 8, 11, and 12, dealing with the amount of sample, automation and miniaturization, waste generation, multianalyte and multiparameter possibilities, reagent toxicity, and operator safety, respectively. In addition, it is important to note that the optimized method (Figure 6A) performed from good to excellent (color changing from yellow to green) in at least 10 of the 12 principles of green analytical chemistry (principles 1, 2, 4, 5, 6, 7, 8, 9, and 12), while the titrimetric methods (Figure 6B and 6C) showed less principles with performance ranging from good to excellent.

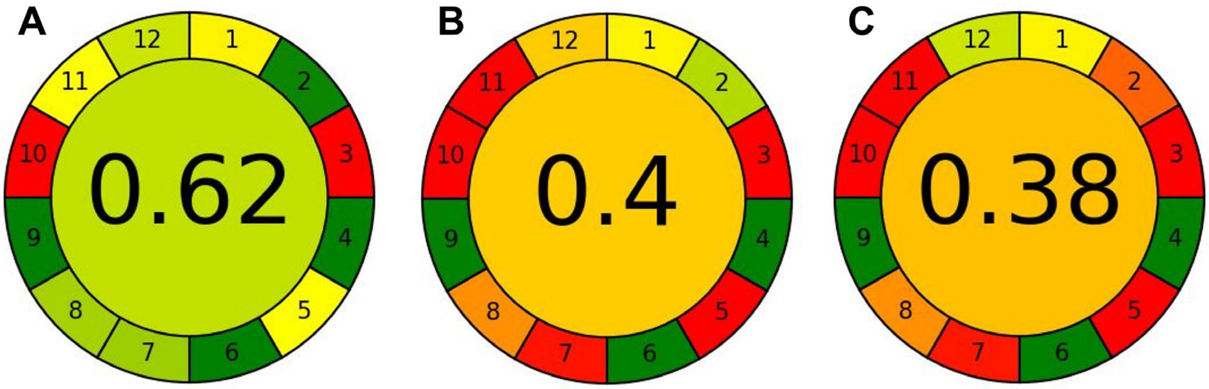


Figure 6. Characteristics of the methods for the determination of basic nitrogen in diesel oil according to green chemistry principles using the Analytical GREEnness Calculator for: (A) miniaturized method developed in this work, (B) potentiometric, and (C) colorimetric titration methods, described in the UOP 269-10 standard method.

CONCLUSIONS

To contribute to the context of nitrogen compounds in fuels, a miniaturized method for colorimetric titration of basic nitrogen fraction in diesel oil was developed using a portable system and digital image acquisition combined with the PhotoMetrix PRO app. Conditions for the neutralization reaction between the basic nitrogen compounds and $HClO_4$ as titrating agent were optimized with crystal violet as indicator. The developed method proved to be advantageous in relation to the methods described in the UOP 269-10 standard method, since it was possible to reduce the consumption of sample and reagents, minimize waste generation, aggregating simplicity in obtaining results. The method developed showed to be suitable to use in routine laboratories, besides the feasibility of use a low-cost system combined with the aspects of green chemistry. The proposed approach could be easily implemented in refineries' quality control laboratories and might contribute to the advances in fuel characterization.

Conflicts of interest

The authors declare no conflict of interest. The founders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Supplementary Material

For the miniaturized method development optimization, the calibration curve was preparing directly in the measurement flask. The total volume inside the flask was maintained in 1120 µL and was adjusted with acetic acid. For this, it was calculated the exact volume needed of each reagent to maintain the concentration according to the evaluated parameter. In general, the volumes used can be visualized in Table S1.

To perform the indicator concentration evaluation, for example, the added volumes ranged from 30 to 120 µL and to maintain constant the total volume, it was altered the acetic acid volume. The same strategy was used for all parameters evaluation.

Table S1. General volumes add for calibration curve preparation in the optimized condition

Basic nitrogen concentration (mg kg^{-1})	Reagent volumes adds (µL)				Total Volume µL
	70 mg kg^{-1} nitrogen standard	Acetic acid	Indicator	$HClO_4$ 20 mmol L^{-1}	
0	0	1040.0	60	20	1120
0.1	1.6	1038.4	60	20	1120
0.5	8	1032.0	60	20	1120
1	16	1024.0	60	20	1120
2	32	1008.0	60	20	1120
3	48	992.0	60	20	1120
4	64	976.0	60	20	1120
5	80	960.0	60	20	1120

(continues on the next page)

Table S1. General volumes add for calibration curve preparation in the optimized condition (continuation)

Basic nitrogen concentration (mg kg^{-1})	Reagent volumes adds (µL)				Total Volume µL
	70 mg kg^{-1} nitrogen standard	Acetic acid	Indicator	$HClO_4$ 20 mmol L^{-1}	
6	96	944.0	60	20	1120
7	112	928.0	60	20	1120
8	128	912.0	60	20	1120
9	144	896.0	60	20	1120
10	160	880.0	60	20	1120