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Short Communication

Validation of LED spectrofluorimeter for determination of both biodiesel and nontransesterified residual cooking oil in diesel samples

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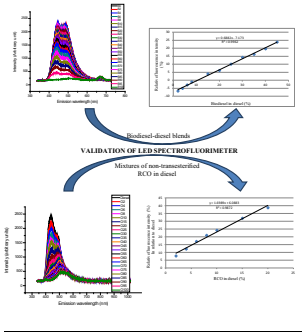
HIGHLIGHTS

- This paper presents the validation of a LED spectrofluorimeter.
- LED spectrofluorimeter was adequate for quantification of biodiesel and RCO in diesel.
- Validation parameters were determined from the regression lines.
- For the analysis of RCO in diesel, the linear range was from 2 to 20% with detection limit of 2%.
- For the analysis of biodiesel in diesel, the range was from 2 to 45% with detection limit of 3%.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper presents the results of the validation of a LED spectrofluorimeter patented for the analysis of biodiesel in diesel and non-transesterified residual cooking oil (RCO) in diesel. Detection limit, quantification limit and sensitivity were determined from the regression lines. The spectrofluorimeter validated in this study was adequate for quantifying the amount of biodiesel in diesel in the range from 2% to 45% (B02–B45) with an *R*-squared value of 0.9962 and a detection limit of 3%. For the analysis of non-transesterified RCO in diesel, the linear range was from 2% to 20% with an *R*-squared value of 0.9872 and a detection limit of 2%. The accuracy of the equipment for the analysis of biodiesel in diesel and non-transesterified RCO in diesel was evaluated using Student's *t*-test for paired data. With 95% confidence level there was no significant difference between the actual values and those determined by the equipment.

Introduction

The analytical method development or adaptation of a known method to a new instrument typically requires a sequence of processes called validation. Validation should demonstrate that the new method or the new equipment meets the requirements of the intended analytical applications and ensures the reliability of the results [1–9].

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The main parameters that are usually part of a validation program are: Linearity, linear range, sensitivity, precision (repeatability), accuracy, detection limit and quantification limit [1].

Linearity is the ability of the method to obtain results directly proportional to the concentration of analyte in the sample within a range specified by the analytical curve [2]. Estimation of the coefficients of the straight line can be made by linear regression. In addition to the regression coefficients angular (a) and linear (b), it is also possible to calculate the correlation coefficient (R) which estimates the quality of the curve obtained. The closer to 1 or −1, the lower the dispersion of the set of experimental points and lower the uncertainty in the estimated regression coefficients. The minimum acceptable correlation coefficient (R) is 0.99. A correlation coefficient of greater than 0.999 is considered as evidence of an optimal adjustment data for the regression line. The squared correlation coefficient is called R-Square or Determination Coefficient defines the percentage of variance of the variables which can be explained from the value of another. R-square of greater than 0.999 means a perfect fit and higher the efficiency of determining the variables y from the x. The coefficient of determination R-square ranges from 0 to 1. The sensitivity can be expressed by the slope of the calibration curve. The sensitivity can be defined as the response varies with the variation in concentration of the analyte.

The range of linearity of the method is the range between the limits of upper and lower quantification [2]. The linearity range is established by confirming that the method provides accuracy, precision and linearity appropriate when applied to samples containing amounts of analyte within the specified range. For any quantitative method, there is a range of concentrations of the analyte in which the method can be applied. However, some analytical procedures do not show linearity. In these cases the analytical response can be described by a different function that models the analyte concentration in the sample.

The accuracy of an analytical method is the closeness of the results obtained in relation to the true value. The main methods for the study of accuracy are based on the use of certified reference material or to compare the proposed method with a reference method.

The precision can be evaluated by the repetitiveness that represents the degree of concordance between the results of successive measurements of the same method, performed under the same measurement conditions, that is, same procedure, same analyst, same instrument used under the same conditions, even local reps in a short time interval. In general, the accuracy is established with a minimum of nine determinations involving a minimum of three different concentration levels. For example, in triplicate assays for three concentration levels.

The Detection Limit (DL) is the lowest amount of analyte which can be detected though not necessarily quantified, under the experimental conditions established. It is usually established through analysis of solutions of known concentrations of analyte and decreasing to the lowest detectable level. In the case of instrumental methods the limit of detection may be estimate based on the ratio of 3 times the baseline noise. DL can be determined by the equation: $DL = 3s_b/a$ where s_b = standard deviation of the background and a = slope of the calibration curve [2]. It can also be determined from the parameters of the regression line. In the latter case the term s_b is the standard error of the term b of the regression line ($y = ax + b$).

The Limit of Quantification (LOQ) is the smallest amount of analyte that can be determined with acceptable precision and accuracy under the experimental conditions established. It is determined through analysis of solutions containing decreasing concentrations of the analyte to the smallest determinable level with acceptable precision and accuracy. Can be determined by the equation: $QL = 10s_b/a$ where s_b = standard deviation of the background and a = slope of the calibration curve [2]. In an analytical curve is usually the lowest point and should not be determined by extrapolation.

The objective of this study was to validate an LED spectrofluorimeter, which was developed and patented by our team [10,11], the technology for which was transferred to the company Quimis S. A. This was then marketed as the Model Q-798FIL for the analysis of biodiesel–diesel blends and was used to determine the adulteration of diesel by non-transesterified residual cooking oil (RCO) instead of biodiesel.

Methodology

The equipment used in this validation study was a prototype of a spectrofluorimeter which uses a quartz cuvette of 1 cm and one violet LED centered at 400 nm as the source for excitation. The emission range was 335–1018.92 nm at intervals of 0.38. Blends of biodiesel and diesel and blends of non-transesterified RCO in diesel were made in triplicate and each batch was analyzed in a Quimis spectrofluorimeter Model Q-798FIL.

Standards of biodiesel in diesel and non-transesterified RCO in diesel were prepared in concentrations (%) of: 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100. The sample of biodiesel was provided by Petrobras and was primarily composed of soy biodiesel. The sample of non-transesterified RCO consisted of residual frying oil of restaurants, which included animal fats, vegetable oils, food, refuse and other organic material. Samples of non-transesterified RCO were filtered to extract the solid residues before being blended with diesel.

Measurements were performed without the cuvette being removed. The cuvette was then washed with the sample itself before the reading was taken. The measurements were taken with the violet LED centered at 400 nm. The measurements of standard samples were taken in triplicate from lower concentrations to higher concentrations.

The measurements of the intensity of the spectra of mixtures of biodiesel and diesel and RCO in diesel were determined at 482 nm and the relative fluorescence intensity values were calculated. The detector used was a monochromator based on CCD (Charge Coupled Device) and a diffraction grating.

Relative fluorescence intensity is the percentage variation of the fluorescence intensity of each spectrum with respect to the fluorescence intensity of the diesel spectrum. This relative change (δ) in percentage was calculated as: $\delta(\%) = (I_0 - I_i)/100I_0$, where I_0 and I_i are respectively the fluorescence intensity of diesel and blends at 482 nm.

Two curves were constructed using the concentrations and relative fluorescence intensity values, with one being for mixtures of biodiesel in diesel and the other for mixtures of RCO in diesel.

Using Excel software, the linear regression equations were found using the concentrations of mixtures of biodiesel in diesel in the range of 2–45% (B02–B45) and concentrations of mixtures of RCO in diesel in the range of 2–20%.

The validation parameters determined were: detection limit, quantification limit, sensitivity and repeatability.

The accuracy was assessed by comparing the actual values of the concentrations of biodiesel (or non-transesterified RCO) in diesel with the predicted values obtained from the respective regression equations. The actual values were added theoretically based in concentration of the mixtures prepared.

Results and discussion

Fig. 1 shows the spectra of biodiesel–diesel blends in the range of 0–100% (B00–B100) obtained with the LED spectrofluorimeter with no correction, that is, without subtracting the intensity of