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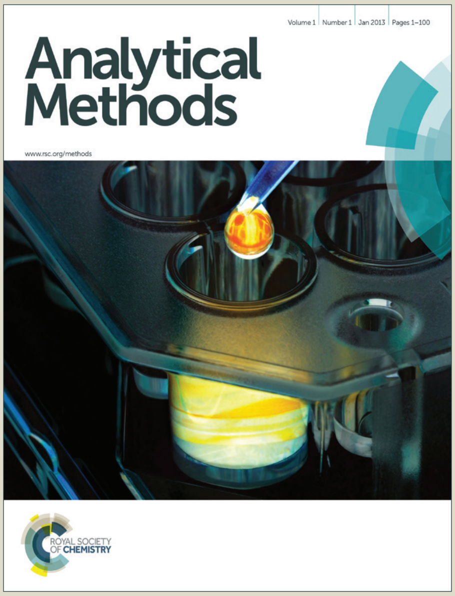

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Greener procedures for biodiesel quality control

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Abstract

Biodiesel analysis often requires large amounts of chemicals and organic solvents with consequent generation of large waste volumes. However, several environmentally friendly alternatives have been presented for assay of biodiesel quality control parameters. These approaches include direct analysis with reagentless procedures, improved sample preparation by dilution or analyte extraction with non-toxic solvents, preparation of emulsions or microemulsions, and sample mineralisation under mild conditions and strategies for minimization of reagent consumption as well as waste generation. These greener alternatives are critically reviewed, highlighting the advantages and limitations of each approach in biodiesel analysis. Alternative analytical techniques (*e.g.* flow analysis and electrochemical detection), predictive studies, and measurement of physicochemical parameters are also discussed.

Keywords: biodiesel; biofuel, green chemistry, green analytical chemistry.

Abbreviations:

AAS: Atomic absorption spectrometry; **AFS:** Atomic fluorescence spectroscopy; **ANP:** Brazilian National Agency of Petroleum, Natural Gas and Biofuels; **ANN:** Artificial neural network; **ASTM:** American Society for Testing and Materials; **ATR:** Attenuated total reflectance; **CN:** Cetane number; **CV:** coefficient of variation; **CV-AFS:** Cold vapour atomic fluorescence spectroscopy; **CFPP:** Cold filter plugging point; **ETAAS:** Electrothermal atomic absorption spectroscopy; **ETV:** Electrothermal vaporization; **FAAS:** Flame atomic absorption spectrometry; **FAES:** Flame atomic emission spectrometry; **FAME:** Fatty acid methyl esters; **FBMN:** Flow Blurring® multinebulizer; **FIA:** Flow injection analysis; **GAC:** Green analytical chemistry; **GC**: gas-chromatography; **HPLC:** high performance liquid-chromatography; **HR-CS ETAAS:** High-resolution continuum source electrothermal atomic absorption spectrometry; **ICP-MS:** Inductively coupled plasma mass spectrometry; **ICP:** Inductively coupled plasma; **ICP OES:** Inductively coupled plasma optical emission spectrometry; **ICP-MS:** Inductively coupled plasma mass spectrometry; **ICP-MS/MS:** Inductively coupled plasma tandem mass spectrometry; **IR:** Infrared spectroscopy; **LLE:** liquid–liquid extraction; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **MCFA:** Multicommutation in flow analysis; **MIR:** Mid infrared spectroscopy; **N_2-MIP OES:** Microwave-induced nitrogen plasma optical emission spectrometry; **MLR:** multiple linear regression; **NIR:** Near infrared spectroscopy; **NMR:** Nuclear magnetic resonance; **PCA:** Principal component analysis; **PDSC:** pressurized differential scanning calorimetry; **PLSR:** Partial least squares regression; **SIA:** Sequential injection analysis; **SPE:** Solid-phase extraction; **UV:** Ultraviolet; **WCAES:** Tungsten coil atomic emission spectrometry.

1. Introduction

Biodiesel is a renewable fuel used in several countries, such as Brazil, Argentina, the United States, Germany, and France. Biodiesel is generally produced by catalytic transesterification (usually with a strong base) of vegetable oils (or animal fats) using methanol (or ethanol). The mono-alkyl esters of fatty acids produced in the reaction can be used in the pure form or blended with petroleum diesel in different proportions. An important characteristic is that physicochemical properties of the product, *e.g.* molar mass, viscosity, and cetane number (CN) are similar to those of petroleum diesel, thus making the use of the existing motors and fuel distribution infrastructure feasible. Biodiesel is also more suitable for use as a fuel than crude vegetable oil because the esters do not cause engine deposits and clogging of injector nozzles.[1] Biodiesel is also a green fuel in view of the low toxicity and low contents of sulphur compounds, as well as the absence of aromatic species. In Brazil, addition of 7% (v/v) biodiesel to commercial diesel is compulsory, and this fraction is anticipated to increase in the near future, which should considerably increase the demand for biofuel.

In view of the widespread use of biodiesel, threshold limits for components and impurities of biodiesel have been established in Brazil,[2] the European Union, and the United States;[3] the main quality parameters and the threshold limits are listed in Table 1. These include the reagents (methanol, ethanol, acylglycerols, hydroxide, acids, and alkaline metals), products of biodiesel synthesis (esters and glycerol) and treatment (*e.g.* water), and raw material impurities (*e.g.* metal ions, P, and S). Quality control also includes additives, such as antioxidants. In addition, the composition of biodiesel may change during storage (*e.g.* due to water absorption, oxidation, and solubilisation of metals from the storage tanks). The regulatory threshold limits tend to be even more restrictive, aiming to assure the product quality, and maximise the fuel yield and motor lifetime, while minimising the environmental impact. Fast and reliable analytical procedures are thus required to reduce adulterations and unconformities.

Recently, several analytical procedures have been developed for determination of the quality parameters of biodiesel, some of which are adaptations of existing strategies for oil analysis. Several general review articles have covered protocols for biodiesel analysis,[1,4,5] whereas others have focused on chromatographic separations,[6] infrared spectroscopy,[7] flow-analysis,[8] electrochemical determination of inorganic[9] and organic[10] species, and quantification of metals and metalloids by atomic spectrometry.[11,12] As a contradiction to the objective of using greener fuels, most of these procedures are time-consuming, expensive, and generate large amounts of wastes (usually comprising toxic chemicals and organic solvents). Titrimetric methods,[13] chromatographic separations that require previous chemical derivatisation[14] and elemental analysis after complete sample mineralisation[15] are representative examples.

Green chemistry is defined as “chemical techniques and methodologies that reduce or eliminate the use or generation of feedstocks, products, and by-products that are hazardous to human health or the environment”.[16] Although initially related to organic synthesis, the concepts have been expanded to other areas, and the term “Green Analytical Chemistry (GAC)” was coined to describe more environmentally friendly analytical methodologies, *i.e.* those that ideally do not produce residues or that minimise their amount, the toxicity, or both. The main challenge is to achieve greener procedures without hindering the fundamental analytical features (accuracy and precision) and with minor impact on sample throughput and the cost of analysis. The trend towards GAC is also fuelled by the high costs and time spent in waste treatment. Review articles discussing different approaches for achieving GAC have also been presented.[16-18]

In this context, several environmentally friendly approaches have been proposed for biodiesel quality control, which encompass reagentless procedures, replacement of toxic chemicals and solvents, minimisation of waste generation, analyte extraction under mild conditions, and direct sample analysis. The aim of this review is to highlight these greener approaches and critically evaluate their potential to supersede conventional methods for routine analysis. Differently from the previous reviews[4-6] that focused on the species being determined, this review is structured to highlight the green approaches aiming direct analysis and sample preparation, as well as alternative analytical techniques. Nevertheless, Tables were organized in function of the target species to facilitate the search for a specific procedure. A general overview of these approaches is presented in Fig. 1, and examples of environmentally friendly approaches for the main parameters used in biodiesel quality control are summarised in Table 1.[19-27]

2. General overview

In a survey of the 76 journals categorised as “*chemical, analytical*” in the Web of Science database (in addition to Spectrochimica Acta Part B - Atomic Spectroscopy, Journal of Analytical Atomic Spectrometry, and Current Analytical Chemistry), 405 studies devoted to biodiesel analysis were found; these received 4534 citations (search in February/2015). Most of these articles (93%) were published in journals with impact factors > 1.0. The first report was related to determination of methanol and free glycerol by gas chromatography (GC) with flame ionisation detection,[28] and this technique remains one of the most usual for analysis of organic species in biodiesel. The evolution of publications and citations on biodiesel analysis is shown in Fig. 2. Although a review article published in 2001 discussed some chromatographic and spectrometric approaches for biodiesel analysis, a significant increase in the publications in the field was observed only after 2006, reflecting the large-scale use of biodiesel as a fuel. For example, in Brazil, addition of 2% (v/v) biodiesel to commercial diesel was first permitted in 2004, and after 2008 the commercialisation of diesel/biodiesel blends became mandatory. The extensive use of biodiesel in Brazil, in addition to specific investments from funding agencies, explains the large number of biodiesel-related publications by Brazilian researchers (*ca.* 45%). Researchers from the United States, China, and Spain respectively contributed with 16, 6, and 4% of these articles.

An interesting feature of biodiesel analysis is that determination of classes of compounds (instead of the individual species) usually suffices for quality control.[1] This aspect is highlighted in Table 1, and Fig. 3 shows that metals are the main class of compounds. In addition to Na and K from the hydroxides commonly used as catalysts for the transterification reaction, other metal ions (that generally originate from the feedstocks) need be monitored due to their effect on biofuel performance and environmental restrictions.[29] Because of the high incidence of procedures for metal determination, atomic spectrometry is widely used (Fig. 4). However, because most of the monitored analytes are organic species, chromatography (mainly GC) and molecular spectrometry (UV-Vis, infrared, and to a lesser extent, fluorescence) are predominantly employed (Fig. 4). Several procedures have also been presented for determination of free and bonded glycerol (*e.g.* by chromatographic and electrochemical detection). In addition to the high relevance of these parameters in biodiesel quality control, the effective separation of the target species from the sample matrix via simple extraction also justifies the large number of applications. Thermal methods are also highly incident (16.5%), but most of them are focused on evaluation of thermal stability of biodiesel produced from different raw materials and the effect of additives. Several studies have focused on determination of the physicochemical properties of biofuel and monitoring of the transterification reaction (Fig. 3). Because these applications often exploit infrared spectroscopy (IR), this technique also stands out in the biodiesel analysis (Fig. 4).

The ideal features of analytical procedures for biodiesel analysis have been summarised, and include high sample throughput, high detectability (for the target species at trace levels), reliability, low-cost, and capacity for simultaneous or sequential determinations.[1] Nevertheless, as is commonly encountered in a realistic situation, these needs have not been simultaneously achieved, and a compromise of conditions need be taken into account in the selection of a suitable approach. The amount and toxicity of the wastes generated is a fundamental aspect to consider (*e.g.* for conformity to the ISO 14000 requirements). Fortunately, a variety of proposed methods satisfy this need and legislative requirements.

The main approaches for green analysis of biodiesel can be classified under at least one of the following strategies:

1. Reagentless procedures, exemplified by direct measurements employing IR, and certain atomic spectrometric methods. Prediction of sample composition from physicochemical properties (*e.g.* flash point) can be also included in this group;
2. Measurements after sample dilution (usually to decrease the sample viscosity and the amount of matrix components) with a low toxicity solvent, such as ethanol. This strategy is common in atomic spectrometry, especially with inductively coupled plasma optical emission spectrometry (ICP OES), and in some electroanalytical procedures;
3. Green extraction of the analytes for separation from the biodiesel matrix, as exploited in some spectrophotometric, fluorimetric, and electroanalytical procedures. This approach also encompasses sample preparation before analysis using separation techniques (*e.g.* capillary electrophoresis) and flow analysis (including on-line

extraction). Environmentally friendly extractants include water, dilute acids, and aqueous ethanolic or surfactant solutions;

4. Green sample mineralisation by exploiting microwave-assisted decomposition with dilute acids and photochemical processes. In spite of the high energy consumption, the use of sample calcination in elemental analysis can also be considered as an alternative to the conventional wet acid digestion;
5. Minimisation of reagent consumption and waste generation, as commonly observed in flow-based procedures and micro-analysis.

Strategies for multi-parametric determinations in the same sample processing, in-line sample dilutions to reduce the waste volume, and chemometric data treatment to obtain analytical information without extensive sample treatment are also valuable approaches. A more detailed discussion of these strategies is presented in the following sections.

3. Direct analysis

As detailed in Table 1, the official methods for determination of Na, K, Ca, Mg, and P in biodiesel are based on ICP OES[30,31] and flame atomic absorption spectrometry (FAAS).[32-36] For S determination, atomic fluorescence is recommended by the American, Brazilian, and European agencies.[37-39] Ultraviolet spectrometry and wavelength-dispersive X-ray fluorescence are environmentally friendly alternatives that allow direct analysis without waste generation or the use of toxic solvents, but dedicated instruments are required. The ICP OES method is also recommended by the Brazilian agency.[40] The procedures require sample dilution with xylene or kerosene in different proportions depending on the norm, the analyte and the analytical technique (ICP OES or FAAS), but at least 10 mL of organic solvent is consumed per determination. Moreover, the use of organometallic standards in oily medium and the addition of mineral oil for matching the viscosity of reference and sample solutions are recommended for external calibrations. Although these methods are simple with high analytical throughput and their sensitivity is in accordance with the current specifications for biodiesel, some disadvantages have been discussed in the literature, including: (i) the use of organic solvents that require special waste management because of toxicity to humans (xylene and kerosene are potentially carcinogenic) and environmental impacts; (ii) conditions for handling organic solvents are different from those for aqueous solutions, sometimes requiring additional accessories and gases; (iii) the organometallic standards tend to be unstable and more expensive than aqueous inorganic ones.

In view of the previously mentioned drawbacks, direct analysis is the preferred approach for analysis of biodiesel because of the simplicity and high sample throughput. The improved detection limits and minimal risk of contamination are additional advantages of this strategy. However, probably due to matrix effects and higher background signals, there are only a few examples of biodiesel elemental analysis without any pretreatment (Table 2). These studies are discussed below, whereas direct IR analysis is discussed in Section 5.3.

Because sample pyrolysis can minimise matrix effects, the metals in biodiesel have been assayed by direct analysis using electrothermal atomic absorption spectrometry (ETAAS) or electrothermal vaporisation (ETV) coupled with other spectroanalytical techniques. From the perspective of GAC, the main advantage of this strategy is the low sample volume required and its quantitative consumption, which eliminates generation of toxic and harmful wastes. In this sense, the first proposed procedure exploited a solid sampling accessory for P determination where 5.00 mg of biodiesel was directly deposited in the platform.[24] Due to volatilization of the analyte as molecular species at temperatures as low as 300 ºC, Pd in Triton X-100 solution was used as chemical modifier, allowing pyrolysis at 1300 ºC. This approach permitted calibration with aqueous standards thus overcoming the previously mentioned drawbacks. However, the need for the solid sampling accessory may restrict its application. Chemical modifiers have also played an important role in P determination by high-resolution continuum source electrothermal atomic absorption spectrometry (HR-CS ETAAS).[41] Measurements were successfully carried out by direct injection of 15 µL of sample into the atomiser, but matrix-matching with P-free biodiesel was required for external calibration.

A tungsten coil was used as an electrothermal vaporiser coupled to inductively coupled plasma mass spectrometry (ICP-MS) with the advantage of requiring a simpler power source.[42] Sample vapours were mixed with the aerosol flow from the nebulisation chamber. Then, 10 µL of sample was directly introduced onto the tungsten coil with a micropipette, and external calibration with aqueous standards was possible due to optimisation of the heating program. This step also prevented both splashing of the sample (which would be expected due to the lower density and higher viscosity compared to aqueous solutions) and degradation of the tungsten coil due to high carbon content in biodiesel.

The Flow Blurring® multinebulizer (FBMN) has allowed compensation of matrix effects in ICP OES determinations.[43] The turbulent pre-mixing between gas and liquid streams provides a more homogeneous aerosol, with smaller (< 10 µm) and narrow-distributed droplets. Based on this device, the direct analysis of biodiesel and on-line implementation of the standard additions method using aqueous standards have been proposed as protocols that do not require introduction of oxygen into the plasma.[44] Notably, the water from the calibration solutions reduced deposit of carbon on tip of the injector tube and the ICP torch compared with what occurs with the use of organic calibration standards. Subsequently, a new FBMN design that employs two nebulisation nozzles instead of three as in the previous proposals was used for biodiesel elemental analysis.[45] External calibration was carried out with on-line addition of an internal standard. Higher nebulisation gas pressures were achieved for the same flow rate, yielding finer aerosols. Organometallic standards were used, but reference solutions were prepared by dilution in mineral oil; thus, toxic solvents and generation of hazardous residues were avoided.

4. Green sample preparation

The analysis of samples with high viscosity, complexity, and carbon content, such as biodiesel, is an analytical challenge, especially considering the difficulties associated with sample introduction and calibration without matrix effects.[12] Sample pretreatment is then usually necessary and various characteristics should be considered for selection of a suitable approach.[11] This includes simplicity, cost, time spent, sources of contamination, dilution factors, as well as the precision and accuracy of the results. Compliance to GAC requirements is an additional challenge, and some available alternatives are discussed in this section.

4.1. Sample dilution

In order to replace xylene or kerosene with low toxicity solvents and to avoid the need for organometallic standards, the simple dilution of biodiesel samples and aqueous inorganic standards in ethanol prior to elemental analysis via ICP OES was first proposed by dos Santos *et al*.[19] This strategy exploits the mutual solubility of biodiesel and aqueous standards in the solvent, which is inexpensive and widely available. Biodiesel dilution in ethanol is thus considered a greener approach, which is feasible in routine analysis. However, the pioneering work highlighted the need for Y as an internal standard to compensate transport effects and introduction of oxygen into the plasma to decrease the effect of background signals on Ca, P, Mg, K, and Na measurements. Introduction of oxygen into the plasma also prevented deposition of carbon on the torch and destabilisation/extinction of the plasma caused by the introduction of high carbon load solutions. Decreasing the spray chamber temperature to -5 °C is an alternative to the introduction of auxiliary gas.[46] This ingenious strategy facilitates solvent condensation in the spray chamber thereby reducing the amount of organic solvent introduced into the ICP. Although plasma stability was achieved, high background emission signals in the 110 to 200 nm range significantly affected the main emission lines of P and S. The software of the ICP OES instrument was adequate for correction of the background signals.

The recently proposed ICP tandem mass spectrometry (ICP-MS/MS) that utilises an octopole reaction cell located in-between two quadrupole mass analysers was used for determination of S in biodiesel after dilution in ethanol.[47] Improved precision and accuracy were successfully achieved with the isotope dilution method. Dilution in ethanol or methanol has also been exploited in biodiesel analysis by microwave-induced nitrogen plasma optical emission spectrometry (N_2-MIP OES),[48] flame atomic emission spectrometry (FAES),[49] flame atomic absorption spectrometry (FAAS),[50] tungsten coil atomic emission spectrometry (WCAES),[51] HR-CS ETAAS,[52] and potentiometry[53] (Table 3). Sample dilutions ranging from 2- to 200-fold[51,53] were utilised in these studies as a compromise between detectability, suppression of matrix effects and drawbacks caused by the high carbon contents in the samples.

In addition to the advantage of using inorganic standards and less toxic solvents, the use of short chain alcohols (mainly ethanol) for sample dilution improves the sensitivity of techniques with nebulisation-based sample introduction compared to measurements employing aqueous medium. This is attributed primarily to the lower surface tension of these solvents. Furthermore, for techniques employing a flame atomiser (FAAS and FAES), the exothermic combustion of the organic solvent also increases the flame temperature and changes it chemical composition, which may enhance the atomisation efficiency.[54] Due to the volatility and flammability of fuels, reduction of the acetylene supply or increasing the air flow rate is recommended to improve flame stability.[11]

Sample dilution in alcohol has also been exploited in the determination of organic species by molecular spectrometry, volumetry or electroanalytical methods. In this sense, ethanol was used to mediate the formation of a single phase between the biodiesel sample and chromogenic reagents (periodate and acetylacetone) in aqueous solution in the spectrophotometric determination of glycerol.[55] The final mixture should contain at least 85% (v/v) ethanol to yield a single phase, thereby precluding the need for time-consuming analyte extraction and phase separation required in other approaches. However, it is necessary to compensate for radiation absorbed by the (coloured) samples, and this was successfully achieved with spectrophotometric measurements without addition of periodate (thus avoiding formation of the formaldehyde intermediate). The procedure is attractive for *in-situ* analysis, which facilitates fast detection of unconformities. When highly sensitive detection approaches are concerned, sample dilution may be useful to minimize interferences and matrix effects. An example is the electrochemical determination of antioxidants, in which the LOD of 75 nmol L^{-1} allowed a 4000-fold sample dilution before analysis.[56] In a green volumetric procedure for acidity determination,[57] sample dilution in a 50% (v/v) ethanol aqueous solution was used to replace the dilution in the 1:1 (v/v) toluene:isopropanol mixture required in the AOCS Cd 3d-63 method.[57] Again, ethanol was used to put together the analytes (free fatty acids) and the reagent (the potassium hydroxide titrant).

4.2. Emulsions and microemulsions

Development of techniques for biodiesel elemental analysis has primarily focused on strategies for simplifying sample preparation, especially without using toxic reagents and solvents. In this sense, preparation of biodiesel emulsions and microemulsions stands out. An emulsion is a heterogeneous and unstable mixture of two immiscible liquids (water and oil, for example) prepared by mechanical stirring. It is frequently stabilised by an emulsifying agent with amphiphilic characteristics (*e.g.* a surfactant) that interacts with both the water and oil phases.[58] In contrast, a microemulsion is optically transparent, has low viscosity, and is thermodynamically stable due to the smaller size of the droplets (structural dimensions between 5 and 100 nm) and its physicochemical characteristics.[58,59] Hydrophilic, hydrophobic, and intermediate hydrophilicity surfactants are necessary to obtain water-in-oil emulsions, oil-in-water emulsions, and microemulsions, respectively.[59] In addition, a co-solvent or a co-surfactant such as *n*-propanol and *n*-pentanol have been frequently used in microemulsion preparations. For analytical purposes, emulsions and microemulsions have allowed the use of aqueous inorganic standards for calibration and reduction of the amount of organic phase in the mixture compared to sample dilution in organic solvents; the use of micro/emulsions has thus resulted in lower background signals, greater stability of plasmas, and minimised hazards from wastes. Furthermore, biodegradable surfactants also contribute to the eco-friendliness of the procedures.

Table 4 summarises the composition of biodiesel emulsions and microemulsions and presents remarks for procedures that have successfully utilised this strategy for sample preparation for elemental analysis. An emulsion prepared with Triton X-100 and nitric acid was used in the determination of Co, Cu, Fe, Mn, Ni, and V in diesel and biodiesel samples using ETV-ICP-MS.[60] Pd acted as a chemical modifier as well as a carrier for aiding transport of the analyte from the ETV to the plasma; better performance was achieved by injecting Pd in conjunction with each sample aliquot. Mercury speciation by flow injection cold vapour generation coupled to atomic fluorescence spectrometry (FI-CV-AFS) employing oil-in-water emulsions of biodiesel has also been presented.[61] Organic compounds were decomposed by UV-irradiation and Hg vapours were generated using an acidic $SnCl_2$ solution. Oil-in-water emulsions were also used for accurate determination of Ca, Cu, Fe, Mg, Mn, Na, and P by ICP OES with axial and radial views with suitable precision.[62] Calibration with inorganic standards in 6% (m/m) Triton X-100 aqueous solutions using Y as an internal standard was successfully accomplished. Because formic acid can partially dissolve in the organic phase thereby facilitating extraction of the analytes to the aqueous phase, its use in biodiesel emulsion preparation was evaluated.[63] This strategy was successfully employed in the determination of Ca, Cu, Fe, Mn, Mg, Na, K, and Si by ICP OES. Blanks and calibration solutions were prepared by using mineral oil for viscosity matching and Y as an internal standard. Determination of Cd and Hg in biodiesel emulsions was carried out by ETAAS by using a Pd–Mg mixture as a chemical modifier due to volatile characteristics of the analytes.[64] Biodiesel microemulsions are more usual than emulsions probably due to the higher stability of the former. Microemulsions were prepared by mixing biodiesel samples with *n*-propanol and an aqueous acid solution to form a transparent and stable mixture for determination of Na and K determinations by FAES.[65] As both discrete and continuous sample introduction yielded similar LOD values, the former approach is preferred because of the lower sample consumption (100 µL) and waste generation. Based on the phase diagram, formation of a microemulsion from Triton X-100, *n*-pentanol, biodiesel, and aqueous standards was optimised for the determination of K and Na using FAAS.[66] While the microemulsion was stable for up to 3 days, the organometallic standard diluted in xylene could be used only for 3 h. The need for frequent preparation of new sets of standards is also an important source of waste generation. In another approach, a microemulsion prepared in *n*-propanol without surfactant was proposed for determination of Ca, Mg, Na and K by FAAS reducing reagents consumption.[67] Despite the variations on microemulsion compositions, these approaches did not present significant differences on LOD. Additional studies based on microemulsion preparation and AAS detection have also been reported for determination of Ca,[68,69] Mg,[68,69] and Zn.[68]

Simultaneous elemental analyses by ICP OES promote higher sample throughput and reduced waste generation compared to monoelemental analysis (*e.g.* by FAAS). In a procedure for simultaneous determination of Ca, Mg, P, and S in biodiesel microemulsions by ICP OES with axial view, the oxygen gas flow-rate introduced into the plasma was optimised by monitoring the signal-to-background ratio.[70] The summation of the intensities of multiple emission lines of S allowed for better sensitivity and accuracy compared to the conventional monitoring of the main emission line, but without significant improvement in LOD.[25]

Highly sensitive techniques are required for determination of potentially toxic elements in biodiesel, which are commonly found at very low concentrations (µg L^{-1}

levels). Because As-containing compounds are generally highly volatile, sample microemulsification prior to graphite furnace ETAAS determination has been utilised to avoid analyte loss - a major drawback of the conventional wet-acid digestion.[71] By using Pd as a chemical modifier, pyrolysis was carried out at 1200 ºC and LOD in the original sample was 0.3 mg kg^{-1}. The potential of ETAAS (high detectability and ability for *in-situ* sample treatment) was also explored for the development of sensitive methods aimed towards determination of other potential pollutant elements that are emitted during fuel burn, such as Cu[72], Cd,[73-75] Pb,[72,73] Ni,[72,74] and Tl.[73] Different metals (Cd, Co, Cu, Mn, Ni, Pb, Ti, and Zn) were determined in biodiesel microemulsified samples by ICP-MS,[76] and the analytical performance with and without oxygen as an auxiliary plasma gas was compared. Although the plasma remained stable even with the high carbon load, the analytical performance declined when no oxygen was used. ICP-MS was also used for determination of S in biodiesel microemulsions.[77] Argon species naturally present in the plasma ($^{36}Ar^+$, $^{36}ArH^+$, or $^{38}Ar^+$) were explored to minimise the well-known oxygen-based polyatomic interferences ($^{16}O_2^+$ and $^{16}O^{18}O^+$) that affect the main abundant S isotopes (^{32}S and ^{34}S). This strategy, termed the interference standard method, does not require instrumental modification or post plasma reaction.

Microemulsification is also useful in eletroanalytical detection. This is exemplified by the potentiometric determination of K^+ in biodiesel with a glassy carbon electrode modified with $Ni_3[Fe(CN)_6]_2$ nanoparticles.[78] The modified electrode was selective for the analyte and stable in the microemulsion prepared in *n*-propanol medium.

It is important to note that the surfactants most commonly used for emulsion and microemulsion preparations are non-ionic Triton X-100 and Triton X-114 that are both readily biodegradable. Microemulsions prepared using a co-surfactant or a co-solvent, mostly use *n*-propanol that is also biodegradable, has low potential for bioconcentration and show low toxicity to fish and other aquatic organisms. Compared to standard methods, this strategy of sample preparation significantly reduces the waste toxicity and is beneficial because it takes environmental and analyst health concerns into account.

4.3. Green extraction

Sample preparation procedures based on analyte extractions from biodiesel samples are also representative in the literature.[79-88] Such procedures involve transfer of analytes from the biodiesel sample to another immiscible solvent (usually simpler than the sample matrix, non-toxic and less hazardous to the environment); the process is usually assisted by mechanical agitation,[79,80,82,83,85,88] including sonication[84,86,87] or heat[81] followed by centrifugation. The main advantages are the simplicity, low cost, and possibility for preconcentration. Table 5 summarises the extraction with aqueous extractants, thus avoiding toxic solvents and highly concentrated acids. This characteristic makes feasible their adoption in routine analysis.

Extraction induced by emulsion breaking has been recently reported for biodiesel analysis of Ca and Mg by fast sequential FAAS,[89] Cu, Mn, and Ni by ETAAS,[90] and Hg by CV-AAS.[91] Four steps are required in this procedure: emulsion formation, mechanical agitation for analyte extraction, heating for emulsion breaking (*i.e.* phase separation) and aqueous phase collection. This strategy is especially advantageous due to the preconcentration of the analytes in the extract and the possibility for external calibration by simple dilution of inorganic standards in water. Internal standardisation with Co in fast sequential FAAS measurements,[89] optimisation of the heating program in ETAAS,[90] and analyte separation by cold-vapour generation[91] were important aspects to make feasible external calibration without matrix effects or spectral interferences.

Greener procedures for determination of organic species have also exploited liquid-liquid or solid-phase extraction (LLE or SPE). Several applications have utilised extraction of water-soluble species to provide an extract that is more suitable for most analytical techniques. This procedure also allowed separation of organic matter that could cause interferences and matrix effects in the analytical determination. Extraction of sulphate with an aqueous ethanolic solution[92] and of glycerol with water[93] can be cited as examples. On-line extraction in flow-based systems is also feasible, as discussed in Section 5.1. Liquid-liquid microextraction in a flow-based system was also exploited for iodine value determination,[22] as an alternative to the titrimetric procedure that consumes large amounts of organic solvents. Measurements were carried out in the aqueous phase containing the I_3^- reagent. Although aqueous extractants are usually limited to ionic and highly polar analytes, pressurised hot-water extraction[94] could be exploited to expand the range of applications by exploiting the significant changes in solvent polarity with temperature and pressure.

Solid-phase extraction (and mainly solid-phase microextraction) is a greener alternative for sample preparation and this approach was successfully adopted for headspace extraction of methanol from biodiesel before analysis by GC.[23] This strategy aimed to avoid chemical derivatisation to trimethylmethoxysilane, which is more time-consuming and requires N,N-dimethylformamide and N,O-bis(trimethylsilyl)-trifluoroacetamide.[28] Even without chemical derivatisation, the sensitivity was sufficient for methanol determination by considering the threshold limit of 0.2% (m/m).

4.4. Green sample mineralisation

Because of the difficulties encountered when high carbon load solutions are introduced into flames or plasmas and the inflammable residues generated with the use of alcohols and fuel samples, the decomposition of biodiesel is usually a preferred strategy. However, from the GAC point of view, this strategy should be explored only if those previously described are not applicable. Dry and wet biodiesel decomposition procedures are found in the literature. A simple, low cost, and effective procedure for the determination of Na in biodiesel by FAES using dry decomposition was proposed.[95] In this procedure, biodiesel was completely decomposed in a muffle furnace in two steps (250 ºC for 1h and 600 ºC for 4 h) and the ash was dissolved in 1% (v/v) HNO_3. Similar sample pretreatment was carried out for Sn(IV) determination by square-wave anodic stripping voltammetry using a bismuth-film electrode.[96] In both cases, long periods were required for biodiesel decomposition; nevertheless, several samples could be decomposed simultaneously without significant waste generation.

Wet sample decomposition generally requires concentrated acids. For example, a mixture of nitric acid sulphuric acids plus hydrogen peroxide was required to initiate the digestion of 1g of biodiesel in an open digestion block and additional aliquots of nitric acid and hydrogen peroxide were required during the heating program.[97] In microwave-assisted digestion, even with the use of less acid, more hazardous species can be produced during decomposition.[94] Fortunately, dilute nitric acid has been effective in sample preparation by microwave-assisted digestion in several fields, including biodiesel analysis. This strategy is feasible because regeneration of nitric acid occurs in the presence of oxygen, and it can be enhanced by pressurising the closed vessels with oxygen gas[98,99] or alternatively by adding hydrogen peroxide as an auxiliary reagent.[99,100] Among the advantages of decomposition with dilute acids, the better compatibility of the digest for plasma techniques should be highlighted. The low acidity of the digests also reduces the blank values, improves LOD and the overall analytical performance.

Approximately 0.1 g biodiesel was successfully digested via microwave-assisted decomposition using a dilute oxidant mixture comprising 4 mL HNO_3, 2 mL H_2O_2, and 2 mL H_2O for simultaneous assay of metals and non-metals using axial view ICP OES.[101] The residual carbon content estimated at 8% (m/m) indicates the effectiveness of the sample digestion. The same strategy was applied to P and S determination in biodiesel by quadrupole-based ICP-MS.[102] Measurements were carried out by monitoring the oxide and hydroxide ions (PO^+, SO^+ and SOH^+) and accuracy was assured by employing the interference standard method. More recently, S, P, and Si were determined in biodiesel by ICP-MS/MS using the mass shift mode.[103] This method overcome spectral interferences and present an instrumental LOD of 0.02 µg L^{-1} S. Isotope dilution sector field-ICP-MS was used for determination of S in biodiesel and low sulphur diesel after microwave-assisted decomposition with dilute nitric acid and hydrogen peroxide. Because of the high accuracy and precision, this method was used for assignment of the S concentration in a certified reference material (SRM 2723b S in diesel fuel oil) in conjunction with data from X-ray fluorescence spectrometry.[104]

Despite the advantages of reduced carbon content and the suitability of the acid digests for the analytical measurements, sample throughput is lower when microwave assisted digestion is utilised compared to direct analysis, simple dilution in alcohol or emulsion and microemulsion preparations.

5. Alternative techniques

5.1. Flow analysis

Flow analysis is a valuable tool for GAC[18,105] that facilitates the development of reagentless procedures, replacement of toxic reagents, minimisation of reagent consumption, and in-line waste treatment. This potential has been also demonstrated for biodiesel analysis,[8] as highlighted in the applications listed in Table 6.

Although some works have successfully exploited flow injection analysis (FIA) in the simplest configuration (*i.e.* single line manifolds),[26,106,107] relatively complex sample treatment is also feasible, as demonstrated in the methanol determination. A sequential injection analysis (SIA) system was designed for membrane-mediated LLE of the analyte, enzymatic conversion to hydrogen peroxide by immobilised alcohol oxidase, and spectrophotometric determination by reaction with 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) catalysed by peroxidase in solution.[108] In spite of the number of steps and incomplete reactions and processes (*e.g.* 20% efficiency of the LLE), good precision (CV < 4.5%) was achieved in view of the highly reproducible timing and reaction conditions. This procedure can be considered an environmentally friendly alternative due to the minimised reagent consumption (see Table 6).

Most applications of flow analysis to biodiesel samples have focused on free glycerol determination with off-line[93,106,107,109] or on-line analyte extraction.[21,26,110] The response range of all of these applications conformed to the legislative threshold value (Tables 1 and 6). These greener procedures are thus reliable alternatives to the conventional approach based on gas chromatography after chemical derivatisation and dilution in organic solvent, which is time-consuming and requires highly toxic reagents (Table 1).[14]

Aiming at a greener procedure, glycerol extraction with water[93] was carried out with the same efficiency achieved with hexane (4 mL per sample) plus ethanol.[111] This strategy was further adopted in other works[107,109] but an additional advantage was the on-line extraction in flow-batch systems,[21,110] which also significantly diminished the sample consumption (*e.g.* from 1000[93,107,109] to 15.4 mg[21,110]) and waste generation (*e.g.* from 8[111] to 1[21] mL) per determination. The flow-batch approach is particularly suitable for this task because the sample/extractant interaction is maximised by magnetic stirring in the chamber; however, a similar effect was achieved in a single line manifold by the joint effect of flow reversal and sonication.[26] Some drawbacks of on-line analyte extraction include variations in the sampled volume with sample viscosity (mainly when time-based sampling is exploited) and the low representativeness of a restricted sample amount (only a few microlitres are required per extraction).

Spectrophotometric[26,93] and fluorimetric[109,110] procedures have used periodate for oxidation of glycerol to formaldehyde and formic acid. Formaldehyde is then determined in the form of 3,5-diacetyl-1,4-dihydrolutidine after reaction with acetylacetone in ammoniacal medium. In this sense, the flow-based approaches significantly reduced the reagent consumption, which was up to 108- (periodate) and 22-fold (acetilacetone)[110] lower than in the analogous batch procedure.[111] On the other hand, in a FIA system with continuous reagent addition,[26] the amount of reagent consumed was 18 (periodate) to 80% (acetylacetone) higher than in the batch method, thus illustrating the need for selection of a suitable approach to effectively achieve a greener procedure. Other green approaches have exploited amperometric detection in alkaline medium to avoid consumption of toxic chemicals.[106,107] The more environmentally friendly alternative is a reagentless procedure based on quenching of the sonoluminescence originating from water-cavitation in the presence of glycerol, after its on-line aqueous extraction.[21] However, the previously mentioned approaches may be preferred over this strategy based on availability of instrumentation.

Bonded glycerol can be converted to free glycerol (*e.g.* by saponification of mono-, di-, and tri-acylglycerols with ethanol in alkaline medium) and determined by the strategies previously described.[26,109] The acylglycerols are then determined by the difference between the total and free amounts. While the batch saponification is time-consuming (30 min was required for quantitative conversion in an ultrasound-assisted process),[109] a flow-based procedure allowed exploitation of incomplete reaction due to the inherently reproducible timing and the higher amount of the analytes in biodiesel.[26] Indeed, sample dilution is often necessary in total glycerol determination and the waste volume can be significantly reduced when this task is carried out on-line.[109]

Water in biodiesel is generally determined by the Karl Fischer titration, which requires specialised equipment, toxic reagents, and rigorous sample handling. Moreover, the accuracy of this method can be lessened by side reactions with additives and impurities usually found in the samples. A greener approach was thus proposed by exploiting colour fading of a cobalt chlorocomplex in ethanolic medium.[27] The standard additions method successfully avoided matrix effects, enabling sample analysis without any pretreatment. Reagent consumption and waste generation (only 750 μL per determination) were minimised by using air as the sample carrier and by intermittent addition of the reagent only in the sample zone, exploiting multicommutation (MCFA). The air carrier also minimised sample dispersion thus achieving the threshold limit recently reduced by Brazilian regulations. The detectability was also improved by using a long path-length flow cell (30 cm optical path).

Air was also exploited as a carrier for introduction of the biodiesel sample (diluted in xylene) for determination of Mn, V, Si, and Cu by ICP OES.[112] In addition to constrain sample dispersion and to reduce solvent consumption, this approach precluded the deleterious effects of large amounts of organic solvents in the plasma. A chamber heated to 350 ^{o}C that was placed before the nebuliser yielded transport efficiency close to 100% and make feasible calibration with a single reference solution by variation of the injected volume.

Free fatty acids are determined in a variety of samples by time-consuming titrimetric procedures in non-aqueous medium; such methods generally consume large amounts of organic solvents. Flow-based titration in a SIA system with spectrophotometric detection was thus proposed as a greener alternative for acidity determination in biodiesel.[113] The procedure exploits the overlap between sample and titrant zones (*i.e.* the pH gradient) and the colour changes provided by an acid-base indicator (alizarin), with multivariate calibration. The procedure yielded results in agreement with the reference procedure without the use of toxic solvents; only 88 μg of the indicator was consumed and only 3.3 mL of waste was generated. However, in spite of the goal to minimise personal errors by automation, CV as high as 21% were still obtained for some samples.

The highly reproducible conditions for sample handling and timing provided by flow analysis were exploited in a green procedure for iodine value determination.[22] A reagent aliquot (aqueous triiodide; 167 μL) was inserted between two aliquots of biodiesel (total volume of 233 μL) without any pretreatment and measurement was based on the fading of the reagent colour due to the halogenation of the unsaturated fatty acids. Significantly improved sample throughput was achieved and the consumption of organic solvents of the titrimetric procedure was avoided (15 mL of carbon tetrachloride and 25 mL of acetic acid were required per determination).[13]

To achieve chemical speciation, photochemical degradation of methyl and ethylmercury was carried out in a FIA system in which a polytetrafluoroethylene reactor was irradiated with a 400 W Hg-lamp.[61] In spite of the mild reaction conditions and the potential for green sample decomposition, photochemical processes are hindered by the high content of organic matter in biodiesel, which has limited further applications. Thus, elemental analysis has often been carried out after mineralisation of biodiesel samples and processing the digests in flow-based systems. This occurs because the high temperatures and time (and eventually the acid concentrations) required for mineralisation of the sample matrix are incompatible with flow-based operation. Thus, in spite of the general trend towards minimising reagent consumption and waste generation, the required sample pretreatment sometimes produces large amounts of waste. This can be exemplified by the spectrophotometric determination of Si, P, Fe, and Al, in which the multicommuted stepwise injection analysis system allowed for less waste generation, but pre-digestion of the sample with 5 mL of 10 mol L^{-1} HNO_3 plus 2 mL of 9 mol L^{-1} H_2O_2 was required.[114] In this sense, a greener alternative was proposed for P determination,[115] involving replacement of the wet-acid digestion in a heating block (which consumes 10 mL HNO_3, 2 mL H_2SO_4 and 6 mL H_2O_2 per determination)[15] by calcination, which requires only 500 mg MgO_2 and an equivalent amount of 1.4 mL H_2SO_4 per determination.[116] Moreover, by using a micro-flow batch system, reagent consumption and waste generation in the spectrophotometric determination of phosphate generated by sample pretreatment were 31- and 15-fold lower than in an FIA system employing continuous reagent addition. Such reductions were achieved by using a 100 μL lab-made micro-chamber built in urethane-acrylate and solenoid micropumps for precise solution handling.

In spite of being time-consuming and requiring high energy consumption, calcination was also exploited for sample preparation in the determination of total S by means of conversion to sulphate.[117] Quantification was carried out in a flow system employing diffuse reflectance spectrometry by exploiting the highly reproducible formation of barium sulphate suspensions. However, because biodiesel generally has a low content of S in the organic form, the determination of free sulphate (from the sulphuric acid used in the transterification reaction) could be exploited to evaluate the product quality. This was successfully carried out in a SIA system after analyte extraction with an ethanol:water (25:75) solution.[92] Indeed, sample analysis without any pretreatment may be feasible.

Although batch injection analysis is not considered a flow-based technique, it shows similar characteristics, such as generation of transient signals and high sample throughput. This strategy was successfully applied for determination of antioxidants after sample dilution in ethanol, as discussed in the next section.[56]

5.2. Electrochemical detection

Electroanalysis generally does not require chemical derivatisation, thus showing a potential for environmentally friendly analytical applications. This also holds for biodiesel analysis,[9,10] as demonstrated by determination of metal ions, acidity, phosphate and organic species. As a general rule, electrochemical detection is characterised by high sensitivity and the main efforts have been to improve the selectivity and avoid matrix effects in the analysis of species of interest in biodiesel. This has been achieved by proper selection of electrode materials (*e.g.* Au,[107,118] Cu,[106] and mainly glassy carbon[56]), electrolytes, working potentials (for voltammetric/ amperometric techniques), and suitable sample pretreatment. Boron-doped diamond[119] is a relatively recently developed electrode-material characterised by low background currents (thus yielding better detection limits) and being less susceptible to adsorption effects, which is advantageous for analysis of samples with high contents of organic species, such as biodiesel.[10] In some applications, only sample dilution (exploiting the inherently high detectability)[56] or simple analyte extraction has sufficed to avoid matrix effects. The standard additions method is also a useful alternative. One drawback is electrode passivation due to the large amounts of organic species present in biodiesel samples. This can be suitably circumvented by using pulsed amperometry,[107] where a suitable potential for dissolution of species deposited at the electrode surface is selected. Pulsed-amperometry also shows potential for simultaneous determinations.[56,119]

Electrochemical detection is typically more cost-effective than most spectrometric and separation techniques, also holding potential for the development of fast and simple procedures. Applications to inorganic species have focused on the determination of Cu,[53] K,[78,79,120] P,[83,85,88] Sn,[96] Ca,[121] and the acid number.[122] The main limitation of the proposed methods is the monoelement character, with the exception of certain amperometric procedures and contactless conductometric detection coupled with capillary electrophoresis.[82]

Given the numerous spectrometric procedures for determination of metal ions in biodiesel, quantification of organic species is a more relevant analytical task. However, electrochemical detection has been restricted to determination of glycerol and antioxidants, especially by amperometry. While some procedures for glycerol determination involve complex sample treatment (*e.g.* analyte extraction, sample clean-up by SPE, and solvent evaporation),[123] other approaches are based on aqueous extracts. Examples include electrocatalytic oxidation of the analyte on a copper electrode in 1 mol L^{-1} NaOH medium[106] and the use of pulsed-amperometry to achieve increased sensitivity and a 10-fold reduction of the hydroxide concentration.[107] Green determination of glycerol in biodiesel has successfully been achieved via separation techniques utilising electrochemical detection coupled to separation techniques also succeed in the green determination of glycerol in biodiesel. HPLC with detection by pulsed-amperometry[124] and capillary electrophoresis with contactless conductivity detector[82] avoided the need for chemical derivatisation that is required in GC determination. Capillary electrophoresis exploited the indirect determination (*i.e.* after reaction of glycerol with periodate to generate iodate). This can be considered a green alternative in view of the simultaneous determination of other organic (formate, acetate, and propionate) and inorganic (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, and PO_4^{3-}) species in the same run, with negligible reagent consumption and waste generation.

Antioxidant additives are inherently electroactive and thus amperometric detection is clearly feasible. Applications include determination of butyl-hydroxytoluene,[118] butyl-hydroxyanisole,[56,119] and mainly tert-butyl-hydroquinone[56,119] after sample dilution in an electrolyte in aqueous/alcoholic (methanol, ethanol, or isopropanol) medium that may contain a surfactant to improve the inherently low solubility of phenolic compounds in water. The approaches that employ only ethanol are the greener alternatives.[56,119] An interesting approach is batch injection analysis, which is based on injection of a sample aliquot directly onto the surface of the working electrode surface, which is immersed in a large-volume of electrolyte. This strategy was successfully applied to the simultaneous determination of butyl-hydroxyanisol and tert-butyl-hydroquinone by means of pulsed-amperometry, requiring only sample dilution in ethanol and ethanol/water/electrolyte solution (40 and 100-fold, respectively).[56]

5.3. Infrared spectrometry

Most methods for biodiesel analysis employ well-established instrumental techniques and are able to provide results with satisfactory precision and accuracy. However, the procedures are generally time-consuming, costly, and not readily amenable to on-line monitoring.[5,7] Alternatively, IR is faster and less laborious and has been applied for biodiesel analysis with the possibility of in-situ measurements with acceptable precision and accuracy. From the perspective of GAC, IR methods are non-destructive and usually do not require reagents and sample pretreatment. The applications of both near-infrared spectroscopy (NIR) or mid-infrared (MIR) spectroscopy involve biodiesel feedstock selection, transesterification reaction monitoring, determination of biodiesel levels in blends, analysis of biodiesel properties, and determination of contaminants.[7]

IR has been widely utilised in association with multivariate calibration for analysis of quality parameters of biodiesel including determining iodine value,[125,126] viscosity,[125,127] cold filter plugging point (CFPP),[125,126] density,[125,127] oxidative stability,[128,129] acid number,[128] and the water,[127,128,130] methanol,[127,130,131] ester, and glycerol content of biodiesel.[20,131]

An important advantage of IR is the possibility of simultaneous multi-parametric determination without complex sample pretreatments or the requirement for different techniques. Although the standard methods are not necessarily destructive or require sample pretreatment, as in the case of determination of density by the hydrometer method,[132] the sample volumes are considerably higher than in IR analysis. Furthermore, reagents and solvents are frequently required in the standard methods, such as in the determination of the iodine value by redox titration,[13] which requires sodium thiosulfate, potassium iodate, and sulphuric acid as well as glacial acetic acid and Wijs reagent for sample pretreatment.

NIR has also been employed for determination of the methanol and water content of biodiesel without employing reagents or solvents – a clear advantage in comparison with conventional methods.[130] Methanol can be determined by the standard method employing headspace GC,[133] while the reference method for water determination is the coulometric titration with several drawbacks (Table 1).[134] Simultaneous determinations of the iodine value, CFPP and fractional composition[126] or kinematic viscosity, density, methanol, and water contents[127] were also possible by employing NIR spectroscopy in association with the artificial neural network (ANN) approach, in which approximately 1 mL of sample was required for each measurement.

MIR has also been employed in the determination of biodiesel properties in combination with multivariate calibration. The performance was compared with that of NIR for monitoring the water content, acid number, and oxidative stability index of biodiesel.[128]

Oxidative stability is an important parameter in biodiesel quality control, which is mainly affected by prolonged storage periods and depends on the raw fat or oil from which the fuel originated. Biodiesel has lower oxidative stability than petroleum diesel and this fuel property should be taken into account because the use of degraded fuel may damage automotive engines.[135-137] The standard method for determination of the oxidative stability and biodiesel acidity require large sample volumes, producing significant amounts of chemical wastes. The Rancimat equipment requires 3.0 g of biodiesel for each measurement,[138] while for determination of the acid number, *ca.* 15 g of sample is dissolved in 50 mL of ethanol/diethyl ether solution before titration with potassium hydroxide.[139] A 1.0 mm optical-path quartz flow cell and an attenuated total reflectance (ATR) probe were used in the NIR and MID measurements, respectively, allowing non-destructive measurements with low sample consumption. The oxidative stability of biodiesel was also determined by NIR spectroscopy in combination with principal component analysis (PCA) and partial least squares regression (PLSR)[129] and by near infrared emission spectroscopy.[140] These methods offered advantages compared to the Rancimat method, including operational simplicity, faster analysis, and lower sample consumption.

Visible spectroscopy and NIR (wavelength range from 400 to 2500 nm) were employed for assay of methanol and glycerol traces in biodiesel samples in combination with modified PLSR.[131] Analysis of glycerol by the GC reference method requires derivatisation with toxic reagents and internal standardisation (1,2,4-butanetriol and tricaprin).[14] It takes 15 min at room temperature followed by dilution in *n*-heptane.[141] The spectroscopic method required only 10 µL of biodiesel without any pretreatment for each spectrum acquisition.

Table 7 summarises various applications of IR for determination of the properties of biodiesel. Despite the advantages previously discussed, the development of multivariate methods involves reliable calibration, requiring a large number of samples to build robust models. In fact, after the model has been established by means of a chemometric tool, spectral acquisition is rapid compared with the several minutes or hours necessary in the standard methods.

Methods using chemometric tools have also been considered for evaluating the similarity of samples of biodiesel and its blends with diesel. Although these methods are not directly related with parameters for evaluation of the quality of the biofuel, they are useful to classify the biodiesel based on the feedstock[142-147] or to check the biodiesel content and adulteration in diesel/biodiesel blends.[148-151] IR has been the most widely used technique for this purpose,[142,143,145,147-150] although other techniques such as GC,[144,146] NMR,[151] and UV-vis and fluorescence spectrometries[147] in have also been used.

5.4. Other alternatives

In addition to the previously mentioned IR methods,[128,129] other greener alternatives for determination of the oxidative stability and other physicochemical parameter of biodiesel have been proposed in the literature. Table 8 summarises selected alternative and environmental friendly methods to this aim.

Spectrofluorimetry in conjunction with multivariate data analysis (PCA and PLSR) was presented as an alternative method. Similar to the IR methods, this strategy requires a large number of samples for the calibration. However, after establishing the model, the determination can be performed in a non-destructive way and without sample pretreatment; the time required for both the spectrofluorimetric measurement and the PLSR is about 20 min.[152] UV-Vis spectrometry was also used to monitor the oxidative stability of biodiesel. Biodiesel degradation was promoted by ultrasonic-accelerated oxidation and the induction time determined was in accordance with Rancimat method.[153]

Measurements of the oxidative stability of biodiesel can be performed by means of pressurised differential scanning calorimetry (PDSC).[154,155] The induction time is estimated from the time required to obtain an exothermic reaction, when a run is carried out under isothermal conditions. On the other hand, the oxidation temperature is obtained from an exothermic peak observed when the run is executed under non-isothermal conditions.[156] The results obtained by PDSC agreed with the oxidation stability index described previously.[157] In addition, compared with the Rancimat method, PDSC can be executed in a shorter period and with a smaller sample amount.

The overall physical and chemical properties of biodiesel depend on the individual fatty esters present in the fuel.[158,159] Thus, the properties of biodiesel samples can be estimated from the chemical composition then avoiding time-consuming experimental runs with the use of large sample and reagent volumes. In the CN determination by the standard method,[160] for example, measurements in a compression ignition diesel engine under set test conditions are necessary in a time-consuming and expensive process and engine calibration should be performed against the blends of two reference fuels (*n*-cetane and hepta-methylnonane).

The determined fatty acid composition or the mass fraction was employed for predicting the viscosities of biodiesel samples from the individual viscosities of the respective fatty acid esters.[161-163] This approach yielded good predictive values, such as an average error of ± 3% when a logarithmic mixture equation was employed for predicting the viscosity of biodiesel from five feedstocks.[161]

The fatty acid methyl ester (FAME) content was also used to define models for prediction of the cloud point,[164,165] iodine value, and saponification value,[166] while the degree of unsaturation and molecular weight of FAME were used to develop four empirical correlations and to estimate CN, higher heating value, density, and viscosity.[167] The average absolute deviations, employed for comparison of the calculated and experimental values, were 5.95%, 0.21%, 0.11%, and 2.57%, respectively.[167] The degree of unsaturation and the chain length were also employed for predicting the density or CN.[168-170] Given that the fatty acid composition of the base oil principally affects the CN of biodiesel, prediction of this parameter was also proposed by employing the ANN,[171,172] with the possibility to obtain a model with an accuracy > 92%.[171]

Iodine value can be estimated from the number of double bonds and the average molecular weight of FAME calculated from ^{1}H Nuclear Magnetic Resonance (NMR) spectra[173] with good correlation with the titrimetric method (r^2= 0.997). ^{1}H NMR was also employed for determination of the iodine value using 1,4-dioxane as an internal standard, allowing analysis with smaller samples than required for the iodometric method.[174] Furthermore, structural data for biodiesel samples derived from ^{1}H NMR spectra combined with multivariate techniques also allowed to infer the CN.[175]

The strategy of determination of one quality parameter from another is interesting to save reagents, sample, and time. Towards this end, a correlation between the flash point and residual alcohol content of biodiesel samples was found and an analytical method for determination of ethanol or methanol was proposed.[176] The alcohol content in biodiesel is currently determined by GC, a more expensive and non-environmentally friendly method.

In fact, prediction of biodiesel quality parameters via modelling from available published data may result in greener strategies, mainly by avoiding experimental runs. However, models are often preceded by determination of the composition of several biodiesel samples using techniques such as HPLC,[164] GC-MS,[167] or ^{1}H NMR,[173] which increase analysis costs.

6. Final considerations

More than 70 procedures devoted to biodiesel analysis that allow reliable measurements without waste generation, or at least reducing the waste volume and hazards (most of them being also fast and cost-effective) were discussed in this review.

The main challenge is to expand the tools discussed in this overview to the determination of other quality parameters and simultaneously simplify the experimental work. Metrological evaluation of these recently proposed approaches are also need to assure that reliable results will be obtained.

Methods that meet all the prerequisites for GAC are scarce. However, greener alternatives, when compared with the standard and traditional methods, have been presented for determination of various parameters for quality control of biodiesel. The determination of inorganic contaminants has been performed by atomic spectrometric techniques subsequent to improved sample pretreatment employing emulsion/ microemulsion preparation, sample dilution with environmentally friendly solvent, extraction with non-toxic solvents, or mineralisation under mild conditions. An interesting trend, observed in more recent works, is towards the use of direct analysis avoiding sample dilution and reducing contamination risks.

Other reagentless procedures can be cited as greener alternatives to biodiesel characterisation. IR can be highlighted as a non-destructive technique that facilitates analysis of contaminants and the properties of biodiesel samples. Another trend is the association of IR with multivariate calibration and chemometric tools for simultaneous multi-parametric determination.

Prediction of the properties of biodiesel from the chemical composition also circunvents time-consuming experimental runs with the use of large sample and reagent volumes. Another interesting strategy that seems to be an opportunity for research is the estimative of one or more quality parameter from another.

Flow analysis is also an important tool for GAC, facilitating the development of procedures with in-line biodiesel sample pretreatment or derivatisation, thereby reducing reagent consumption and waste generation.

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References

1. G. Knothe, *Trans. ASAE*, 2001, **44**, 193–200.
2. ANP Resolution n. 14. 2012. Establishes the minimum specifications to be observed for commercialization of biodiesel in whole Brazilian territory. http://www.anp.gov.br. Accessed in November/2014.
3. ASTM D6751. 2011. Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels. http://www.astm.org. Accessed in November/2014.
4. M. R. Monteiro, A. R. P. Ambrozin, L. M. Lião and A. G. Ferreira, *Talanta*, 2008, **77**, 593–605.
5. I. P. Lobo, S. L. C. Ferreira and R. S. Cruz, *Quim. Nova*, 2009, **32**, 1596–1608.
6. R. E. Pauls, *J. Chromatogr. Sci.*, 2011, **49**, 384–393.
7. W. B. Zhang, *Renew. Sust. Energ. Rev.*, 2012, **16**, 6048–6058.
8. V. R. B. Soares, F. R. P. Rocha and L. S. G. Teixeira, *Anal. Lett.*, 2013, **46**, 1621–1639.
9. A. L. Santos, R. M. Takeuchi, R. A. A. Munoz, L. Angnes, N. R. Stradiotto, *Electroanal.*, 2012, **24**, 1681–1691.
10. A. L. Santos, R. M. Takeuchi, R. A. A. Munoz, L. Angnes and N. R. Stradiotto, *Electroanal.*, 2014, **26**, 233 – 242.
11. M. G. A. Korn, D. S. S. dos Santos, B. Welz, M. G. R. Vale, A. P. Teixeira, D. C. Lima and S. L. C. Ferreira, *Talanta*, 2007, **73**, 1–11.
12. R. Sánchez, C. Sánchez, C. P. Lienemann and J. L. Todolí, *J. Anal. At. Spectrom.*, 2015, **30**, 64–101.

13. European Standard EN 14111: Fat and oil derivatives - Fatty acid methyl esters (FAME) - Determination of iodine value, CEN - European Committee for Standardization, Brussels, Belgium, 2003.
14. ASTM D6584-07, Test method for the determination of free and total glycerin in B 100 biodiesel methyl esters by gas chromatography, ASTM International.
15. L. F. B. Lira, D. C. M. B. dos Santos, M. A. B. Guida, L. Stragevitch, M. G. A. Korn, M. F. Pimentel and A. P. S. Paim, *Fuel*, 2011, **90**, 3254–3258.
16. P. T. Anastas, *Crit. Rev. Anal. Chem.*, 1999, **29**, 167–175.
17. S. Armenta, S. Garrigues and M. de la Guardia, *Trends Anal. Chem.*, 2008, **27**, 497–511.
18. F. R. P. Rocha, L. S. G. Teixeira, J. A. Nóbrega, *Spectrosc. Lett.*, 2009, **42**, 418–429.
19. E. J. dos Santos, A. B. Herrmann, E. S. Chaves, W. W. D. Vechiatto, A. C. Schoemberger, V. L. A. Frescura and A. J. Curtius, *J. Anal. At. Spectrom.*, 2007, **22**, 1300–1303.
20. P. Baptista, P. Felizardo, J. C. Menezes and M. J. N. Correia, *Anal. Chim. Acta*, 2008, **607**, 153–159.
21. P. H. G. D. Diniz, M. F. Pistonesi, M. C. U. Araújo and B. S. F. Band, *Talanta*, 2013, **114**, 38–42.
22. A. C. Pereira and F. R. P. Rocha, *Anal. Chim. Acta*, 2014, **829**, 28–32.
23. M. C. Paraschivescu, E. G. Alley, W. T. French, R. Hernandez, K. Armbrust, *Bioresour. Technol.*, 2008, **99**, 5901–5905.
24. F. H. Lyra, M. T. W. D. Carneiro, G. P. Brandão, H. M. Pessoa and E. V. R. de Castro, *J. Anal. At. Spectrom.*, 2009, **24**, 1262–1266.
25. C. G. Young, R. S. Amais, D. Schiavo, E. E. Garcia, J. A. Nóbrega and B. T. Jones, *Talanta*, 2011, **84**, 995–999.
26. S. Pinzi, F. Priego Capote, J. Ruiz Jiménez, M. P. Dorado, M. D. Luque de Castro, *Bioresour. Technol.*, 2009, **100**, 421–427.
27. A. C. Pereira, B. F. Reis and F. R. P. Rocha, *Talanta*, 2015, **131**, 21–25.
28. M. Mittelbach, G. Roth and A. Bergmann, *Chromatographia*, 1996, **42**, 431-434.
29. Y -F. Wang, K. -L. Huang, C. -T. Li, H. -H. Mi, J. -H. Luo and P. -J. Tsai, *Atmos. Environ.*, 2003, **37**, 4637–4643.

30. DIN EN 14538. (2006) *Fat and Oil Derivatives—Fatty Acid Methyl Esters (FAME), Determination of Ca, K, Mg and Na Content by Optical Emission Spectral Analysis with Inductively Coupled Plasma (ICP OES).*
31. EN 16294 Petroleum Products And Fat And Oil Derivatives - Determination of Phosphorus Content In Fatty Acid Methyl Esters (Fame) - Optical Emission Spectral Analysis With Inductively Coupled Plasma (ICP OES).
32. BS EN 14109. (2003) *Fat and Oil Derivatives—Fatty Acid Methyl Esters (FAME), Determination of Potassium Content by Atomic Absorption Spectrometry*.
33. BS EN 14108. (2003) *Fat and Oil Derivatives—Fatty Acid Methyl Esters (FAME), Determination of Sodium Content by Atomic Absorption Spectrometry*.
34. ABNT NBR 15554:2008. Produtos derivados de óleos e gorduras. Ésteres metílicos/etílicos de ácidos graxos. Determinação do teor de sódio por espectrometria de absorção atômica. *Fat oil and derivatives. Fatty acid methyl esters. Determination of the sodium, potassium, calcium and magnesium contents by atomic absorption spectrometry*.
35. ABNT NBR 15555:2008. Produtos derivados de óleos e gorduras. Ésteres metílicos/etílicos de ácidos graxos. Determinação do teor de potássio por espectrometria de absorção atômica *Fat oil and derivatives. Fatty acid methyl esters. Determination of the sodium, potassium, calcium and magnesium contents by atomic absorption spectrometry*.
36. ABNT NBR 15556:2008. Produtos derivados de óleos e gorduras. Ésteres metílicos/etílicos de ácidos graxos. Determinação do teor de sódio, potássio, cálcio e magnésio por espectrometria de absorção atômica. *Fat oil and derivatives. Fatty acid methyl esters. Determination of the sodium, potassium, calcium and magnesium contents by atomic absorption spectrometry*.
37. DIN EN ISO 20846 (2011) - *Petroleum products - Determination of sulphur content of automotive fuels -- Ultraviolet fluorescence method.*
38. DIN EN ISO 20884 (2004) - *Petroleum products - Determination of sulphur content of automotive fuels -- Wavelength-dispersive X-ray fluorescence spectrometry.*
39. ASTM D5453 (2012) - *Standard Test Method for Determination of Total Sulphur in Light Hydrocarbons, Spark Ignition Engine Fuel, Diesel Engine Fuel, and Engine Oil by Ultraviolet Fluorescence.*

40. ABNT NBR 15867 (2010) Biodiesel - Determinação do teor de enxofre por espectrometria de emissão óptica com plasma indutivamente acoplado (ICP OES). *Determination of S by Optical Emission Spectral Analysis with Inductively Coupled Plasma (ICP OES).*

41. R. C. Campos, C. L. T. Correia, F. Vieira, T. D. Saint' Pierre, A. C. Oliveira and R. Gonçalves, *Spectrochim. Acta Part B*, 2011, **66**, 352–355.

42. G. L. Donati, R. S. Amais and J. A. Nóbrega, *J. Anal. At. Spectrom.*, 2013, **28**, 280–287.

43. M. A. Aguirre, N. Kovachev, B. Almagro, M. Hidalgo and A. Canals, *J. Anal. At. Spectrom.*, 2010, **25**, 1724–1732.

44. M. A. Aguirre, N. Kovachev, M. Hidalgo and A. Canals, *J. Anal. At. Spectrom.*, 2012, **27**, 2102–2110.

45. F. D. A. Orozco, N. Kovachev, M. A. A. Pastor, C. E. Domini, B. S. F. Band and A. C. Hernández. *Anal. Chim. Acta*, 2014, **827**, 15–21.

46. E. S. Chaves, M. T. C. de Loos-Vollebregt, A. J. Curtius and F. Vanhaecke, *Spectrochim. Acta Part B*, 2011, **66**, 733–739.

47. L. Balcaen, G. Woods, M. Resano and F. Vanhaecke, *J. Anal. At. Spectrom.*, 2013, **28**, 33–39

48. R. S. Amais, G. L. Donati, D. Schiavo and J. A. Nóbrega, *Microchem. J.*, 2013, **106**, 318–322.

49. A. I. Barros, A. P. Oliveira, M. R. L. Magalhães and R. D. Villa, *Fuel*, 2012, **93**, 381–384.

50. M. R. L. Magalhães, A. I. Barrosa, A. P. Oliveira, A. S. Silva and R. D. Villa, *Current Anal. Chem.*, 2014, **10**, 166–171.

51. S. E. Dancsaka, S. G. Silva, J. A. Nóbrega, B. T. Jones, G. L. Donati, *Anal. Chim. Acta*, 2014, **806**, 85–90.

52. D. P.C. Quadros, M. Rau, M. Idrees, E. S. Chaves, A. J. Curtius and D. L. G. Borges, *Spectrochim Acta Part B*, 2011, **66**, 373–377.

53. E. S. Almeida, M. A. N. A. Monteiro, R. H. O. Montes, R. Mosquetta, N. M. M. Coelho, E. M. Richter and R. A. A. Muñoz, *Electroanal.*, 2010, **22**, 1846–1850.

54. B. Welz and M. Sperling, *Atomic Absorption Spectrometry*, Wiley-VCH, New York, 3th edn, 2005, ch. 8, pp. 341.

55. M. S. Ribeiro, F. R.P. Rocha, *Microchem. J.*, 2013, **106**, 23–26.

56. T. F. Tormin, R. R. Cunha, E. M. Richter and R. A. A. Munoz, *Talanta*, 2012, **99**, 527–531.

57. J. A. Aricetti and M. Tubino, *Fuel*, 2012, **95**, 659-661.

58. C. Viana, D. Bohrer, L. M. Carvalho, P. C. Nascimento and M. B. da Rosa, *Trends Anal. Chem.*, 2014, **53**, 49–59.

59. J. L. Burguera and M. Burguera, *Talanta*, 2004, **64**, 1099-1108.

60. E. S. Chaves, F. G. Lepri, J. S. A. Silva, D. P. C. Quadros, T. D. Saint'Pierre and A. J. Curtius, *J. Environ. Monit.*, 2008, **10**, 1211–1216.

61. P. R. Aranda, P. H. Pacheco, R. A. Olsina, L. D. Martinez and R. A. Gil, *J. Anal. At. Spectrom.*, 2009, **24**, 1441–1445.

62. R. M. Souza, L. G. Leocádio and C. L. P. Silveira, *Anal. Lett.*, 2008, **41**, 1615–1622.

63. M. T. Lisboa, C. D. Clasen, D. C. S. Vellar, E. Q. Oreste, T. D. Saint'Pierre, A. S. Ribeiro and M. A. Vieira, *J. Braz. Chem. Soc.*, 2014, **25**, 143–151.

64. P. R. Aranda, J. A. Gásquez, R. A. Olsina, L. D. Martinez and R. A. Gil, *Talanta*, 2012, **101**, 353–356.

65. E. S. Chaves, T. D. Saint'Pierre, E. J. Santos, L. Tormen, V. L. A. F. Bascuñana and A. J. Curtius, *J. Braz. Chem. Soc.*, 2008, **19**, 856–861.

66. A. de Jesus, M. M. Silva and M. G. R. Vale, *Talanta*, 2008, **74**, 1378–1384.

67. F. H. Lyra, M. T. W. D. Carneiro, G. P. Brandão, H. M. Pessoa and E. V. de Castro, *Microchem. J.*, 2010, **96**, 180–185.

68. R. S. Amais, E. E. Garcia, M. R. Monteiro and J. A. Nóbrega, *Fuel*, 2012, **93**, 167–171.

69. A. de Jesus, A. V. Zmozinski, J. A. Barbará, M. G. R. Vale and M. M. Silva, *Energy Fuels*, 2010, **24**, 2109–2112.

70. R. S. Amais, E. E. Garcia, M. R. Monteiro and J. A. Nóbrega, *Br. J. Anal. Chem.*, 2011, **5,** 246–250.

71. M. A. Vieira, L. C. C. de Oliveira, R. A. Gonçalves, V. de Souza and R. C. Campos, *Energy Fuels*, 2009, **23**, 5942–5946.

72. F. A. Lobo, D. Goveia, A. P. de Oliveira, E. R. Pereira-Filho, L. F. Fraceto, N. L. Dias Filho and A. H. Rosa, *Fuel*, 2009, **88**, 1907–1914.

73. J. S. A. Silva, E. S. Chaves, E. J. dos Santos, T. D. Saint'Pierre, V. L. A. Frescura and A. J. Curtius, *J. Braz. Chem. Soc.*, 2010, **21**, 620–626.

74. F. A. Lobo, D. Goveia, A. P. Oliveira, L. P. C. Romão, L. F. Fraceto, N. L. Dias Filho and A. H. Rosa, *Fuel*, 2011, **90**, 142–146.

75. A. S. Lima, D. G. Silva and L. S. G. Teixeira, *Environ. Monit. Assess*., 2015, **187**, 4122.

76. R. S. Amais, E. E. Garcia, M. R. Monteiro, A. R. A. Nogueira and J. A. Nóbrega, *Microchem. J.*, 2010, **96**, 146–150.

77. R. S. Amais, G. L. Donati and J. A. Nóbrega, *J. Braz. Chem. Soc.*, 2012, **23**, 797–803.

78. G. C. Sedenho, L. L. Paim and N. R. Stradiotto, *Anal. Methods*, 2013, **5**, 4145–4151.

79. M. S. Castilho and N. R. Stradiotto, *Talanta*, 2008, **74**, 1630–1634.

80. M. Piovezan, A. C. O. Costa, A. V. Jager, M. A. L. de Oliveira and G. A. Micke, *Anal. Chim. Acta*, 2010, **673**, 200–205.

81. M. Ghisi, E. S. Chaves, D. P. C. Quadros, E. P. Marques, A. J. Curtius and A. L. B. Marques, *Microchem. J.*, 2011, **98**, 62–65.

82. T. Nogueira and C. L. do Lago, *Microchem. J.*, 2011, **99**, 267–272.

83. L. Torrezani, A. A. Saczk, M. F. de Oliveira, N. R. Stradiotto and L. L. Okumura, *Electroanal*., 2011, **23**, 2456–2461.

84. L. B. de Caland, E. L. C. Silveira and M. Tubino, *Anal. Chim. Acta*, 2012, **718**, 116–120.

85. T. R. C. Zezza, M. S. Castilho and N. R. Stradiotto, *Fuel*, 2012, **95**, 15–18.

86. S. S. M. Rodrigues, A. S. Lima, L. S. G. Teixeira, M. G. A. Korn and J. L.M. Santos, *Fuel*, 2014, **117**, 520–527.

87. A. S. Lima, S. S. M. Rodrigues, M. G. A. Korn, D. S. M. Ribeiro, J. L. M. Santos and L. S. G. Teixeira, *Microchem. J.*, 2014, **117**, 144–148.

88. M. C. Sartori, T. R. C. Zezza, L. L. Paim and N. R. Stradiotto, *Fuel*, 2014, **117**, 564–567.

89. F. M. Pereira, D. M. Brum, F. G. Lepri and R. J. Cassella, *Microchem. J.*, 2014, **117**, 172–177.

90. F. M. Pereira, R. C. Zimpeck, D. M. Brum, R. J. Cassella, *Talanta*, 2013, **117**, 32–38.

91. P. O. Vicentino, D. M. Brum, R. J. Cassella, *Talanta*, 2015, **132**, 733–738.

92. V. del Río, M. S. Larrechi and M. P. Callao, *Anal. Chim. Acta*, 2010, **676**, 28–33.

93. S. G. Silva and F. R. P. Rocha, *Talanta*, 2010, **83**, 559–564.

94. D. L. Rocha, A. D. Batista, F. R. P. Rocha, G. L. Donati and J. A. Nóbrega, *Trends Anal. Chem.*, 2013, **45**, 79–92.

95. A. P. Oliveira, R. D. Villa, K. C. P. Antunes, A. Magalhães and E. C. Silva, *Fuel*, 2009, **88**, 764–766.

96. M. Frena, I. Campestrini, O. C. Braga and A. Spinelli, *Electrochim. Acta*, 2011, **56**, 4678–4684.

97. M. G. A. Korn, D. C. M. B. Santos, M. A. B. Guida, I. S. Barbosa, M. L. C. Passos, M. L. M. F. S. Saraiva and J. L. F. C. Lima, *J. Braz. Chem. Soc.*, 2010, **21**, 2278–2284.

98. C. A. Bizzi, E. M. M. Flores, R. S. Picoloto, J. S. Barin and J. A. Nóbrega, *Anal. Methods***,** 2010, **2**, 734–738.

99. C. A. Bizzi, J. A. Nóbrega and J. S. Barin, in *Microwave-Assisted Sample Preparation for Trace Element Determination*, ed. E. M. M. Flores, Elsevier, 1st edn., 2001, vol. 1, ch. 6, pp. 187–189.

100. C. A. Bizzi, E. L. M. Flores, J. A. Nóbrega, J. S. S. Oliveira, L. Schmidt and S. R. Mortari, *J. Anal. At. Spectrom.*, 2014, **29**, 332–338.

101. A. N. Nascimento, J. Naozuka, P. V. Oliveira, *Br. J. Anal. Chem.*, 2011, **3**, 131–135.

102. G. L. Donati, R. S. Amais and J. A. Nóbrega, *J. Anal. At. Spectrom.*, 2012, **27**, 1274–1279.

103. R. S. Amais, C. D. B. Amaral, L. L. Fialho, D. Schiavo and J. A. Nóbrega, *Anal. Methods*, 2014, **6**, 4516–4520.

104. R. S. Amais, S. E. Long, J. A. Nóbrega and S. J. Christopher, *Anal. Chim. Acta*, 2014, **806**, 91–96.

105. W. R. Melchert, B. F. Reis and F. R. P. Rocha, *Anal. Chim. Acta*, 2012, **714**, 8–19.

106. A. H. Maruta and T. R. L. C. Paixão, *Fuel*, 2012, **91**, 187–191.

107. T. G. G. Barbosa, E. M. Richter and R. A. A. Munoz, *Electroanal.*, 2012, **24**, 1160–1163.

108. A. R. T. S. Araujo, M. L. M. F. S. Saraiva, J. L. F. C. Lima and M. G. A. Korn, *Anal. Chim. Acta*, 2008, **613**, 177–183.

109. S. G. Silva, A. Morales-Rubio, M. de la Guardia and F. R. P. Rocha, *Anal. Bioanal. Chem.*, 2011, **401**, 365–371.

110. M. B. Lima, M. Insausti, C. E. Domini, M. F. Pistonesi, M. C. U. Araújo and B. S. F. Band, *Talanta*, 2012, **89**, 21–26.

111. P. Bondioli and L. Della Bella, *Eur. J. Lipid Sci. Technol.*, 2005, **107**, 153–157.

112. R. Sánchez, J. L Todolí, C. P. Lienemann and J. M. Mermet, *J. Anal. At. Spectrom.*, 2012, **27**, 937–945.

113. V. del Río, M. S. Larrechi, M. P. Callao, *Talanta*, 2010, **81**, 1572–1577.

114. C. S. Vakh, A. V. Bulatov, A. Y. Shishov, A. V. Zabrodin and L. N. Moskvin, *Fuel*, 2014, **135**, 198–204.

115. M. B. Lima, I. S. Barreto, S. I. E. Andrade, M. S. S. Neta, L. F. Almeida, M. C. U. Araújo, *Talanta*, 2012, **98**, 118–122.

116. E. L. C. Silveira, L. B. Caland and M. Tubino, *Fuel*, 2011, **90**, 3485–3488

117. M. Tubino and C. A. R. Queiroz, *Ecl. Quím.*, 2009, **34**, 29 – 36.

118. J. Chylkova, M. Tomaskova, R. Selesovska and J. Jehlicka, *Electroanal.*, 2012, **24**, 1374–1379.

119. R. A. Silva, D. T. Gimenes, T. F. Tormin, R. A. A. Munoz, E. M. Richter, *Anal. Methods*, 2011, **3**, 2804–2808.

120. P. Rapta, J. Paligová, H. Rotheneder and J. Cvengroš, *Chem. Pap.*, 2007, **61**, 337–341.

121. J. M. S. Almeida, R. M. Dornellas, S. Yotsumoto-Neto, M. Ghisi, J. G. C. Furtado, E. P. Marques, R. Q. Aucélio and A. L.B. Marques, *Fuel*, 2014, **115**, 658–665.

122. M. Tubino and J. A. Aricetti, *J. Braz. Chem. Soc.*, 2011, **22**, 1073–1081.

123. L. M. Lourenço, N. R. Stradiotto, *Talanta*, 2009, **79**, 92–96.

124. P. Lozano, N. Chirat, J. Graille and D. Pioch, *Fresenius J. Anal. Chem.*, 1996, **354**, 319–322.

125. P. Baptista, P. Felizardo, J. C. Menezes and M. J. N. Correia, *Talanta*, 2008, **77**, 144–151.

126. R. M. Balabin, *Energy Fuels*, 2011, **25**, 2373–2382.

127. R. M. Balabin, E. I. Lomakina and R. Z. Safieva, *Fuel*, 2011, **90**, 2007–2015.

128. L. F. B. de Lira, M. S. de Albuquerque, J. G. A. Pacheco, T. M. Fonseca, E. H. de Siqueira, L. Stragevitch and M. F. Pimentel, *Microchem. J.*, 2010, **96**, 126–31.

129. N. Canha, P. Felizardo, J. C. Menezes and M. J. N. Correia, *Fuel*, 2012, **97**, 352–357.

130. P. Felizardo, P. Baptista, J. C. Menezes and M. J. N. Correia, *Anal. Chim. Acta*, 2007, **595**(1–2), 107–113.

131. M. P. Dorado, S. Pinzi, A. de Haro, R. Font and J. Garcia-Olmo, *Fuel*, 2011, **90**, 2321–2325.

132. European Standard EN ISO 3675: Crude petroleum and liquid petroleum products – laboratory determination of density – hydrometer method, CEN - European Committee for Standardization, Brussels, Belgium, 1998.
133. European Standard CSN EN 14110: Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of methanol content, CEN – European Committee for Standardization, Brussels, Belgium, 2003.
134. European Standard EN ISO 12937: Petroleum products - Determination of water - Coulometric Karl Fischer titration method, CEN – European Committee for Standardization, Brussels, Belgium, 2000.
135. M. L. M. Valle, R. S. Leonardo and J. Dweck, *J. Therm. Anal. Calorim.*, 2014, **116**, 113–118.
136. A. L. Carvalho, S. M. F. Santana, C. S. Silva, I. M. Pepe, M. A. Bezerra, L. M. Aragão, C. M. Quintella and L. S. G. Teixeira, *J. Braz. Chem. Soc.*, 2013, **24**, 1373–1379.
137. J. Pullen and K. Saeed, *Renew. Sust. Energ.Rev.*, 2012, **16**, 5924–5950.
138. European Standard EN 14112: Fat and oil derivatives - Fatty acid methyl esters (FAME) -Determination of oxidation stability (accelerated oxidation test), CEN – European Committee for Standardization, Brussels, Belgium, 2003.
139. European Standard EN 14104: Fat and oil derivatives - Fatty acid methyl esters (FAME) - Determination of acid value, CEN – European Committee for Standardization, Brussels, Belgium, 2003.
140. F. S. Vieira and C. Pasquini, *Fuel*, 2014, **117**, 1004–1009.
141. European Standard EN 14105: Fat and oil derivatives - Fatty acid methyl esters (FAME) - Determination of free and total glycerol and mono-, di- and triglyceride content, CEN – European Committee for Standardization, Brussels, Belgium, 2011.
142. R. M. Balabin and R. Z. Safieva, *Anal. Chim. Acta*, 2011, **689**, 190–197.
143. S. J. Mazivila, F. B. de Santana, H. Mitsutake, L. C. Gontijo, D. Q. Santos and W. Borges Neto, Fuel, 2015, 142, 222–226.
144. M. E. Flood, J. C. Goding, J. B. O’Connor, D. Y. Ragon and A. M. Hupp, J. Am. Oil Chem. Soc., 2014, 91, 1443–1452.
145. G. Veras, A. A. Gomes, A. C. da Silva, A. L. B. de Brito, P. B. A. de Almeida and E. P. de Medeiros, *Talanta*, 2010, **83**, 565–568.
146. S. P. Schale, T. M. Le and K. M. Pierce, *Talanta*, 2012, **94**, 320–327.

147. M. Insausti, A. A. Gomes, F. V. Cruz, M. F. Pistonesi, M. C. U. Araujo, R. K. H. Galvão, C. F. Pereira and B. S. F. Band, *Talanta*, 2012, **97**, 579–583.

148. M. J. C. Pontes, C. F. Pereira, M. F. Pimentel, F. V. C. Vasconcelos and A. G. B. Silva, *Talanta*, 2011, **85**, 2159–2165.

149. M. F. Pimentel, G. M. G. S. Ribeiro, R. S. da Cruz, L. Stragevitch, J. G. A. Pacheco Filho and L. S. G. Teixeira, *Microchem. J.*, 2006, **82**, 201–206.

150. S. J. Mazivila, L. C. Gontijo, F. B. de Santana, H. Mitsutake, D. Q. Santos and W. Borges Neto, *Energy Fuels*, 2015, **29**, 227–232.

151. M. R. Monteiro, A. R. P. Ambrozin, L. M. Lião, E. F. Boffo, E. R. Pereira-Filho and A. G. Ferreira., *J. Am. Oil Chem. Soc.*, 2009, **86**, 581–585.

152. M. Meira, C. M. Quintella, A. S. Tanajura, H. R. G. da Silva, J. D. S. Fernando, P. R. da Costa Neto, I. M. Pepe, M. A. Santos and L. L. Nascimento, *Talanta*, 2011, **85**, 430–434.

153. F. D. A. Orozco, A. C. Sousa, C. E. Domini, M. C. U. Araújo and B. S. F. Band, *Ultrason. Sonochem.*, 2013, **20**, 820–825.

154. R. O. Dunn, *Trans. ASAE*, 2000, **43**, 1203−1208.

155. R. O. Dunn, *Trans. ASABE*, 2006, **49**, 1633−1641.

156. S. Jain and M. P. Sharma, *Renew. Sust. Energ. Rev.*, 2010, **14**, 1937–1947.

157. R. O. Dunn, *J. Am. Oil Chem. Soc.*, 2005, **82**, 381−387.

158. A. A. Refaat, *Int. J. Environ. Sci. Tech.*, 2009, **6**, 677−694.

159. E. G. Giakoumis, *Renew. Energ.*, 2013, **50**, 858−878.

160. European Standard ISO 5165: Petroleum products – Determination of the ignition quality of diesel fuels - Cetane engine method – European Committee for Standardization, Brussels, Belgium, 1998.

161. C. A. W. Allen, K. C. Watts, R. G. Ackman and M. J. Pegg, *Fuel*, 1999, **78**, 1319−1326.

162. K. Krisnangkura, T. Yimsuwan and R. Pairintra, *Fuel*, 2006, **85**, 107−113.

163. X. Meng, M. Jia and T. Wang, *Fuel*, 2014, **121**, 133−140.

164. H. Imahara, E. Minami and S. Saka, *Fuel*, 2006, **85**, 1666−1670.

165. J. C. A. Lopes, L. Boros, M. A. Krähenbühl, A. J. A. Meirelles, J. L. Daridon, J. Pauly, I. M. Marrucho and J. A. P. Coutinho, *Energy Fuels*, 2008, **22**, 747−752.

166. A. Gopinath, S. Puhan and G. Nagarajan, *Renew. Energ.*, 2009, **34**, 1806−1811.

167. L. F. Ramírez-Verduzco, J. E. Rodríguez-Rodríguez and A. R. Jaramillo-Jacob, *Fuel*, 2012, **91**, 102−111.

168. S. Phankosol, K. Sudaprasert, S. Lilitchan, K. Aryusuk and K. Krisnangkura, *Energy Fuels*, 2014, **28**, 4633−4641.

169. M. Lapuerta, J. Rodríguez-Fernández and O. Armas, *Chem. Phys. Lipids*, 2010, **163**, 720−727.

170. D. Tong, C, Hu, K. Jiang and Y. Li, *J. Am. Oil Chem. Soc.*, 2011. **88**, 415−423.

171. R. Piloto-Rodríguez, Y. Sánchez-Borroto, M. Lapuerta, L. Goyos-Pérez and S. Verhelst, *Energ. Convers. Manage.*, 2013, **65**, 255−261.

172. A.S. Ramadhas, S. Jayaraj, C. Muraleedharan and K. Padmakumari, *Renew. Energ.*, 2006, **31**, 2524−2533.

173. R. Kumar, V. Bansal, M. B. Patel and A. S. Sarpal, *Energy Fuels*, 2012, **26**, 7005−7008.

174. M. Oromí-Farrús, G. Villorbina, J. Eras, F. Gatius, M. Torres and R. Canela, *Fuel*, 2010, **89**, 3489−3492.

175. D. V. Nadai, J. B. Simões, C. E. N. Gatts and P. C. M. L. Miranda, *Fuel*, 2013, **105**, 325−330.

176. H. F. Boog, E. L. C. Silveira, L. B. Caland and M. Tubino, *Fuel*, 2011, **90**, 905−907.

Figure captions

Fig. 1 Schematic representation of the strategies for greener analysis of biodiesel.

Fig. 2 Evolution of publications and citations on biodiesel analysis.

Fig. 3 Distribution of the analytical methods applied to biodiesel by considering the species determined.

Fig. 4 Distribution of the analytical methods applied to biodiesel in function of the analytical techniques.

Table 1 Main parameters for biodiesel quality control

Parameter	Importance of monitoring	Threshold limit	Official method[d]	Drawback	Example of green alternative
Ca + Mg and Na + K	May promote corrosion of the vehicle engine and reduce its performance by soap formation	5 mg/kg[a-c]	FAAS and ICP OES	Sample dilution in xylene or kerosene with large waste generation	Sample dilution in ethanol and determination by ICP OES[19]
Ester	Indicates the efficiency of the transterification reaction	96.5% (m/m)[b,c]	GC	Sample dilution in toluene or heptane	Direct measurement by IR and multivariate calibration[20]
Free glycerol	May cause damage to the engine and hazardous emissions	0.020% (m/m)[a-c]	GC	Chemical derivatisation[e] after sample dilution in heptane	Reagentless flow-based sonoluminescence[21]
Iodine value	Measurement of the total degree of insaturation in biodiesel	120 g I_2/100 g[a-c]	Titrimetry	Large volume of acetic acid and carbon tetrachloride	Flow-based liquid-liquid microextraction[22]
Methanol or ethanol	May cause metal corrosion during fuel storage and use; impair CN, the flash point, and lubricity of the fuel	0.2% (m/m)[b,c]	headspace-GC	Low repeatability for manual sampling	GC after extraction by headspace solid-phase microextraction[23]
P	May damage catalytic converters reducing its efficiency to avoid pollutant emission	10[a,b] and 4[c] mg/kg	ICP OES	Sample dilution in xylene or kerosene with large waste generation	Direct analysis by ETAAS after sample dilution in ethanol[24]
S	May cause environmental impact (*e.g.* acid rain, emission of sulphates particulates) and poison of platinum catalysts	(15[a]; 10[b,c]) mg/kg	UV fluorescence and ICP OES	Dedicated fluorescence instrument; sample dilution in xylene for ICP OES analysis	Determinations by ICP OES in biodiesel microemulsions[25]
Total glycerol	Sum of free and mono, di, and triacylglycerols, which indicates the efficiency of the transesterification reaction	0.25% (m/m) [a-c]	GC	Chemical derivatisation[e] after sample dilution in heptane	On-line saponification and quantification of free glycerol[26]
Water	May lead to biofuel hydrolysis, microorganism proliferation, and alterations in the oxidative stability	200 mg/kg[b]	Coulometric Karl Fischer titration	Sulphur dioxide and iodine dissolved in pyridine and methanol	Flow procedure based on colour fading of the cobalt chlorocomplex[27]

According to **a.** American, **b.** Brazilian and **c.** European norms; **d.** *Ca + Mg:* EN 14538; *Ester:* EN 14103; *Free glycerol:* ASTM D6584, EN 14105 and 14106; *Iodine value:* EN 14111; *Methanol or ethanol:* EN 14110; *P:* ASTM D4951, EN 14107; *Na + K:* EN 14108, 14109 and 14538; *S:* ASTM D5453; *Total glycerol:* ASTM D6584, EN14105; *Water:* ASTM D6304, ISO 12937; **e.** with N,N-dimethylformamide and N,O-bis(trimethylsilyl)-trifluoroacetamide

Table 2 Atomic spectrometric techniques for determination of inorganic contaminants in biodiesel in combination with direct analysis.

Analytes	Method	Reagents	Green aspect	LOD (µg g^{-1})	Ref.
P	GF AAS	20 µL of the Pd 1500 mg mL^{-1} in 0.1% (v/v) HNO_3 and 0.025% (v/v) Triton X-100 as chemical modifier	sample analysis (5.00 mg) without any pretreatment using solid sampling accessory	1.2	24
P	HR-CS GF AAS	Pd (30 µg) and $Mg(NO_3)_2$ (20 µg) dissolved in 0.1% (v/v) Triton X-100 and 0.2% (v/v) HNO_3 was used as modifier	15 µL of sample directly injected into the atomizer	0.5	41
P and Si	ICP-MS	—	Direct analysis by ETV on a tungsten coil	0.4 and 0.1	42
Na, K, Ca, Mg, P, and 20 minor components	ICP OES	—	A three-nozzle FBMN used for direct nebulisation of biodiesel	0.0003-0.03	44
Na, K, Ca, Mg, P, and 20 minor components	ICP OES	1 mg L^{-1} Y as internal standard solution	A two-nozzle FBMN for nebulisation of biodiesel samples and water, avoiding acid digestion	Ca, K, Mg, Na, and P: 0.005-0.5; minor components: 0.001-3	45

Table 3 Selected procedures for elemental analysis of biodiesel based on sample dilution in alcohol.

Analyte	Method	Remarks	Ref.
Ca, P, Mg, K, and Na	ICP OES	Oxygen gas to keep plasma stability and reduce background signal. Y as internal standard improved the precision	19
Ca, Cu, Fe, K, Mg, Na, P, S, and Zn	ICP OES	Cooled spray chamber (−5 °C) reduced the amount of organic solvent introduced into the ICP avoiding carbon deposition on the torch and attenuating spectral interferences	46
S	ICP-MS/MS	Mass shift mode with oxygen as reaction gas and isotope dilution for calibration.	47
Si	N_2-MIP OES	Aqueous calibration by using FBMN. Air injected into the plasma prevented C deposits, improved stability and minimized background signal	48
Na and K	FAES	Washed biodiesel obtained by acid extractions replaced mineral oil in the adjust of standard solutions viscosity	49
Na, K, Ca, and Mg	FAAS	Washed biodiesel obtained by acid extractions replaced mineral oil in the adjust of standard solutions viscosity. Better sensitivity for Na, K and Mg in ethanol in comparison to xylene medium	50
Na, K, Cr, and V	WCAES	Measurements of Na/K and Cr/V in biodiesel diluted in methanol and ethanol, respectively. Higher atomizer power (150 to 250 W) for Cr and V determinations due to its refractory characteristics	51
Al, Cu, Fe, and Mn	HR-CS ETAAS	Platform pre-treated with Zr to prevent carbide formation and improve precision in Al determinations. Calibration with aqueous standards, except for Al that required matrix-matching	52
Cu	Potentiometry	Biodiesel dilution in ethanol-water solution before detection by stripping chronopotentiometry using a gold working-electrode. The standard additions method was necessary for accurate determination	53

Table 4 Biodiesel emulsion and microemulsion composition and remarks of proposed methods for biodiesel elemental analysis.

Analyte	Method	Sample preparation	Composition	Remarks	Ref.
S	ICP OES	Microemulsion	0.5 mL 20% (v/v) HNO_3, 0.5 mL Triton X-100, 2–3mL biodiesel and *n*-propanol up to 10 mL	Summation of the intensities of sulphur multiple emission lines for better sensitivity, and good accuracy	25
Co, Cu, Fe, Mn, Ni, and V	ETV-ICP-MS	Emulsion	1.0 g sample, 2.0 mL Triton X-100 5% (m/v), 0.5 mL HNO_3 and deionized water up to 10 mL	Rh was used as internal standard and Pd (1 µg) as carrier/modifier lead to calibration curves using aqueous solutions and inorganic standards	60
Speciation of Hg	FI-CV-AFS	Emulsion	1 mL biodiesel, 3 mL HNO_3 and 1.5 mL Triton X-100	Organic mercury was decomposed with UV irradiation; Hg vapours were generated using an acidic $SnCl_2$ solution in a continuous flow system	61
Ca, Cu, Fe, Mg, Mn, Na, and P	ICP OES	Emulsion	1.0 g biodiesel, 0.2 mL HNO_3, 0.6 g Triton X-100 and water until 10 g	Better LOQ values in axial view; calibration curves using 6 % Triton X-100 (m/m) aqueous solutions and inorganic standards	62
Ca, Cu, Fe, Mn, Mg, Na, K, and Si	Potentiometry	Emulsion	1.0 g biodiesel, 1.5 mL formic acid, 1.0 mL Triton X-100 1% (v/v), antifoam and water up to 10 mL	Stability of emulsified aqueous standards and samples of at least 3 h; Y internal standard.	63
Cd and Hg	ETAAS	Emulsion	1g biodiesel, 0.25mL HNO_3, Triton X-100 and 1.5 mL isopropyl alcohol	Pd–Mg as chemical modifier (500 mg g^{-1}); Standard addition method for accurate determinations	64
Na and K	ICP OES	Microemulsion	0.5 g biodiesel, 0.4 mL Cs 5% (m/v) solution, 100 µL HCl and *n*-propanol up to 10 mL	Discrete and continuous sample introduction presented similar LOD values; CsCl as ionization suppressor. Base oil for viscosity matching	65
Na and K	FAAS	Microemulsion	57.6% (m/m) *n*-pentanol, 20% (m/m) biodiesel, 14.4% (m/m) Triton X-100 and 8% (m/m) aqueous standard in diluted HNO_3	Base oil used for viscosity matching	66

Table 4 – *cont.*

Analyte	Method	Sample preparation	Composition	Remarks	Ref.
Na, K, Ca, and Mg	FAAS	Microemulsion	1 g biodiesel, 0.1 mL HNO_3, 0.25 mL 2 % (m/v) KCl and 1.15mL 0.2% (v/v) HNO_3, diluted with *n*-propanol to 10 mL.	Organometallic standard solutions	67
Ca, Mg, and Zn	FAAS	Microemulsion	1 mL biodiesel, 0.5 mL Triton X-100, 0.5 mL HNO_3 20% (v/v), and *n*-propanol to complete 10 mL	Sensitivities by using discrete aspiration were up to 7.0-fold better than by continuous aspiration	68
Ca and Mg	FAAS	Microemulsion	0.86 g biodiesel, 0.62 g Triton X-100, and 150 µL water adjusted to 5 mL with 2.6 g *n*-pentanol.	Base oil used for viscosity matching.	69
Ca, Mg, P and S	ICP OES	Microemulsion	0.5 mL 20 % (v/v) HNO_3, 0.5 mL Triton X-100, and 1.0 mL biodiesel and *n*-propanol to 10 mL	Oxygen addition to the auxiliary gas to reduce background signals; external calibration using mineral oil and inorganic standards	70
As	ETAAS	Microemulsion	0.1 g biodiesel, 1 mL of *n*-propanol, 0.2 mL of HNO_3 and *n*-propanol up to 10 mL	2000 mg L^{-1} Pd in 0.2 % (v/v) HNO_3 used as chemical modifier	71
Cu, Pb, Ni, and Cd	ETAAS	Microemulsion	0.5 g biodiesel, 5 g Triton X-100 and HNO_3 1% (v/v) until 50 mL	Chemical modifiers (Pd + Mg and W) and sample preparation procedures (microemulsion and wet digestion) were investigated	72

Table 4 *cont.*

Analyte	Method	Sample preparation	Composition	Remarks	Ref.
Cd, Pb, and Tl	ETAAS	Microemulsion	2 g biodiesel, 1 mL 10% (v/v) HNO_3 and *n*-propanol until 10 mL	Matrix matching was required in calibration for accurate results	73
Ni and Cd	ETAAS	Microemulsion	0.5 g biodiesel, 5 g Triton X-100 and 1% (v/v) HNO_3 until 50 mL	Tungsten was used as a permanent modifier; Co-surfactant was not used	74
Cd	ETAAS	Microemulsion	2.1 mL biodiesel, 2.1 mL HNO_3 and *n*-propanol until 10 mL	External calibration with aqueous standards	75
Cd, Co, Cu, Mn, Ni, Pb, Ti, and Zn	ICP-MS	Microemulsion	0.25 mL Triton X-100, 0.25 mL 20% (v/v) HNO_3, 0.50 mL biodiesel and 4.0 mL *n*-propanol	Oxygen introduced in the composition of auxiliary gas	76
S	ICP-MS	Microemulsion	1.0 mL biodiesel, 0.5 mL 20% (v/v) HNO_3, 0.5 mL Triton X-100 and *n*-propanol up to 10 mL	Interference method to overcome polyatomic interferences	77
K	Potentiometry	Microemulsion	biodiesel:*n*-propanol:aqueous phase phase 5:70:25	Glassy carbon electrode modified with nickel (II) hexacyanoferrate nanoparticles	78

Table 5 Procedures for biodiesel analysis based on green analyte extraction.

Analyte	Method	Extractant (time/min)	Ref.
K	Potentiometry using nickel hexacyanoferrate-modified electrode	HCl 0.1 mol L^{-1} (NI)	79
Na, K, Ca, and Mg	Capillary electrophoresis and UV detection	Water (20)	80
Cu and Fe	GFAAS	25% m/v tetramethylammonium hydroxide at 90±3 °C (5)	81
Ca, K, Mg, Na, SO_4^{2-}, PO_4^{3-}, formate, acetate, propionate, and glycerol	Capillary electrophoresis with capacitively coupled contactless conductivity detection	3 mmol L^{-1} MES/His, pH 6 (0.5)	82
Phosphate	Voltammetry	0.5 mol L^{-1} $NaNO_3$ or 0.5 mol L^{-1} KCl (5 min), followed by resting for 10 min	83
Na, K, Mg, and Ca	Ion chromatography	water or 1.0 mol L^{-1} HNO_3 aqueous solution (1), heating at 85 ºC (30) and sonication (15)	84
P	Cyclic voltammetry using 1:12 phosphomolybdic modified electrode	1.0 mol L^{-1} H_2SO_4 (NI)	85
Fe	Fluorescence quenching of CdTe quantum dots	1:1 (v/v) concentrated HCl and H_2O_2 assisted by sonication (50); heating for H_2O_2 elimination	86
Cu	CdTe-GSH quantum dots as photoluminescence probes	6 mol L^{-1} HCl + 30% (m/m) H_2O_2 assisted by sonication (60); heating for H_2O_2 elimination	87
P	Potenciometry with electrode modified with cobalt film	1.0 mol L^{-1} H_2SO_4 (5)	88
Sulphate	SIA-spectrophotometry	Ethanol:water 25:75 (v/v)	92
Glycerol	Flow-injection spectrophotometry	Water (30) and centrifugation (5)	93

His: L-histidine; MES: 2-(N-morpholino)ethanesulfonic acid; NI: not informed

Table 6 Applications of flow-based procedures for green analysis of biodiesel

Analyte	FA/DET	Sample pretreatment	Green aspect	Reagent consumption (mg)[a]	LR (mg kg^{-1})[b]	CV (%)	SR (h^{-1})	Ref.
Acidity	SIA/SP	Not required	Avoid toxic reagents and minimizes reagent consumption	Alizarine (0.016), NaOH (0.008), ethanol (3)	NI	10[c]	12	113
Antioxidants (BHA,TBHQ)	BIA/AP	Dilution in ethanol and ethanol/water	Avoid toxic reagents	—	NI	1.0	170	56
	MPFS/SP	analyte extraction with water	Greener extraction and minimized reagent consumption	KIO_4 (0.345); acetylacetone (15)	20-200	1.5	34	93
	FB/SL	Not required	Reagentless procedure	—	0.004-400	1.5	14	21
Free glycerol	MPFS/FL	analyte extraction with water	Greener extraction and minimized reagent consumption	KIO_4 (0.19); acetylacetone (13)	20-280	0.9	35	109
	FIA/AP	analyte extraction with water	No toxic chemicals	NaOH (40)	60-3200	5.0	90	106
	FB/FL	Not required	Greener extraction and minimized reagent consumption	KIO_4 (0.025); acetylacetone (1); *isooctane (0.238)*	0.4-20	1.5	14	110
	FIA/AP	analyte extraction with water	No toxic chemicals	NaOH (11)	0.36-110	1.5	85	107
Iodine value	MCFA/SP	Not required	On-line liquid-liquid microextraction	I_2 (0.2)	13-135[d]	1.7	108	22
Methanol	SIA/SP	Not required	Membrane mediated LLE and minimized reagent consumption	ABTS (0.088), *xylene (0.186)*	2-150	4.5	12	108
Hg speciation	FIA/CV-AFS	oil-in-water emulsion with Triton X-100 and HNO_3	Photodegradation of organic compounds containing Hg under mild conditions	Triton X-100 (1.5), HNO_3 (3), $SnCl_2$ (NI)	8-120	< 8.0	NI	61
P	μFB/SP	Calcination at 550 °C	Avoid wet-acid digestion and minimizes reagent consumption and waste generation in the spectrophotometric determination	MgO_2 (0.5), *H_2SO_4 (2.0)*, $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.14), $C_8H_4K_2O_{12}Sb_2.3H_2O$ (0.003), ascorbic acid (1.0)	0.5-50	4.5	189	115
Sulphate	SIA/SP	Extraction with ethanol:water (25:75).	Green extraction and minimized reagent consumption	Ba-DMSA (0.012)	NI	3.3[c]	15	92
Total glycerol	MPFS/FL	saponification with sodium ethylate under sonication	Greener extraction and minimized reagent consumption	NaOH (50); KIO_4 (0.19); acetylacetone (13); *ethanol (2)*	50-600	1.4	35	109
Water	MCFA/SP	Not required	Avoid toxic organic solvents and minimizes reagent consumption	$CoCl_2$ (0.0035), *ethanol (0.75)*	100-5000	0.7	30	27

a. amount per determination – volumes in mL for liquids and solutions (in italic); b. referent to the biodiesel sample; c. mean value estimated from the sample analysis; d. g I_2/100g; ABTS: 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); AMP: amperometry; AP: amperometry; Ba-DMSA: barium dimethylsulphonazo (III) complex; BHA: butylated hydroxyanisole; FL: fluorescence; NI: not informed; SL: sonoluminescence; SP: spectrophotometry; TBHQ: tert-butylhydroquinone

Table 7 Applications of green IR procedures for determining biodiesel properties

Parameter	Spectra region	Chemometric tool	Remarks	Ref.
Ester content	Near	PCR and PLSR	A transflectance probe for determinations with low sample volume	20
Iodine value, CFPP, kinematic viscosity and density	Near	PCA and PLSR	Multi parameter determination with low sample volume	125
Oxidative stability index, acid number and water content	Middle and near	MLR and PLSR	Quick way to monitor the stability of biodiesel during storage	128
Water and methanol contents	Near	PCR and PLSR	Determination without reagents or solvents	130
Density, kinematic viscosity, water and methanol contents	Near	ANN	Multi parameter determination with low sample volume	127
Oxidative stability	Near	PCA and PLSR	A transflectance probe for determinations with low sample volume	129
Fractional composition, iodine value and CFPP	Near	ANN	Multi parameter determination with low sample volume	126
Methanol and glycerol content	Near and visible	MPLSR	10 µL of biodiesel for each spectrum acquisition	131

Table 8 Selected alternative methods for determination of biodiesel properties

Parameter	Strategy/technique	Remarks	Ref.
Oxidation stability	Spectrofluorimetry	Spectrofluorimetry was associated with multivariate data analysis (PCA and PLSR). Determination in 20 min without sample pretreatment	143
Oxidation stability	UV-Vis spectrometry	Sample degradations were induced by ultrasonic-accelerated oxidation. Fast determination of the induction time when compared with Rancimat method	144
Oxidation stability	PDSC	The induction time is estimated from the time required to obtain an exothermic reaction. Fast and require low sample amount when compared with Rancimat method	147
Viscosity	Prediction	Prediction of viscosities from the knowledge of fatty acid composition	151
Viscosity	Prediction	A thermodynamic model was proposed for the determination of kinematic viscosities at different temperatures	152
Viscosity	Prediction	ANN was used to predict the kinematic viscosity with experimental data of FAME composition collected from the literature	153
Cloud point	Prediction	Prediction model was established from a thermodynamic study. Cloud point was estimated by the amount of saturated FAME.	154
Iodine value and saponification value	Prediction	Prediction from a multiple linear regression model obtained from FAME composition	156
Cetane number, kinematic viscosity, density and higher heating value	Prediction	Empirical correlations were proposed to estimate the four physical properties as a function of the degree of unsaturation and molecular weight.	157
Density	Prediction	Empirical equations based on free energy additivity were proposed from fatty acid composition	158
Cetane number	Prediction	MLR model developed for correlation of CN with the fatty acid composition	160
Cetane number	Prediction	Models were proposed from FAME composition using MLR and ANN	161
Cetane number	Prediction	ANN models developed to predict CN from the fatty acid compositions	162
Iodine Value	NRM	^{1}H NMR spectra to determinate the number of double bonds and the average molecular weight of the FAME for calculation of iodine value	163
Cetane number	NRM	Structural information from ^{1}H NMR spectra in association with PCA, fuzzy clustering and a feed-forward ANN used for inference of CN	165
Ethanol or methanol	Flash point	Direct correlation between the flash point and the residual alcohol concentration	166

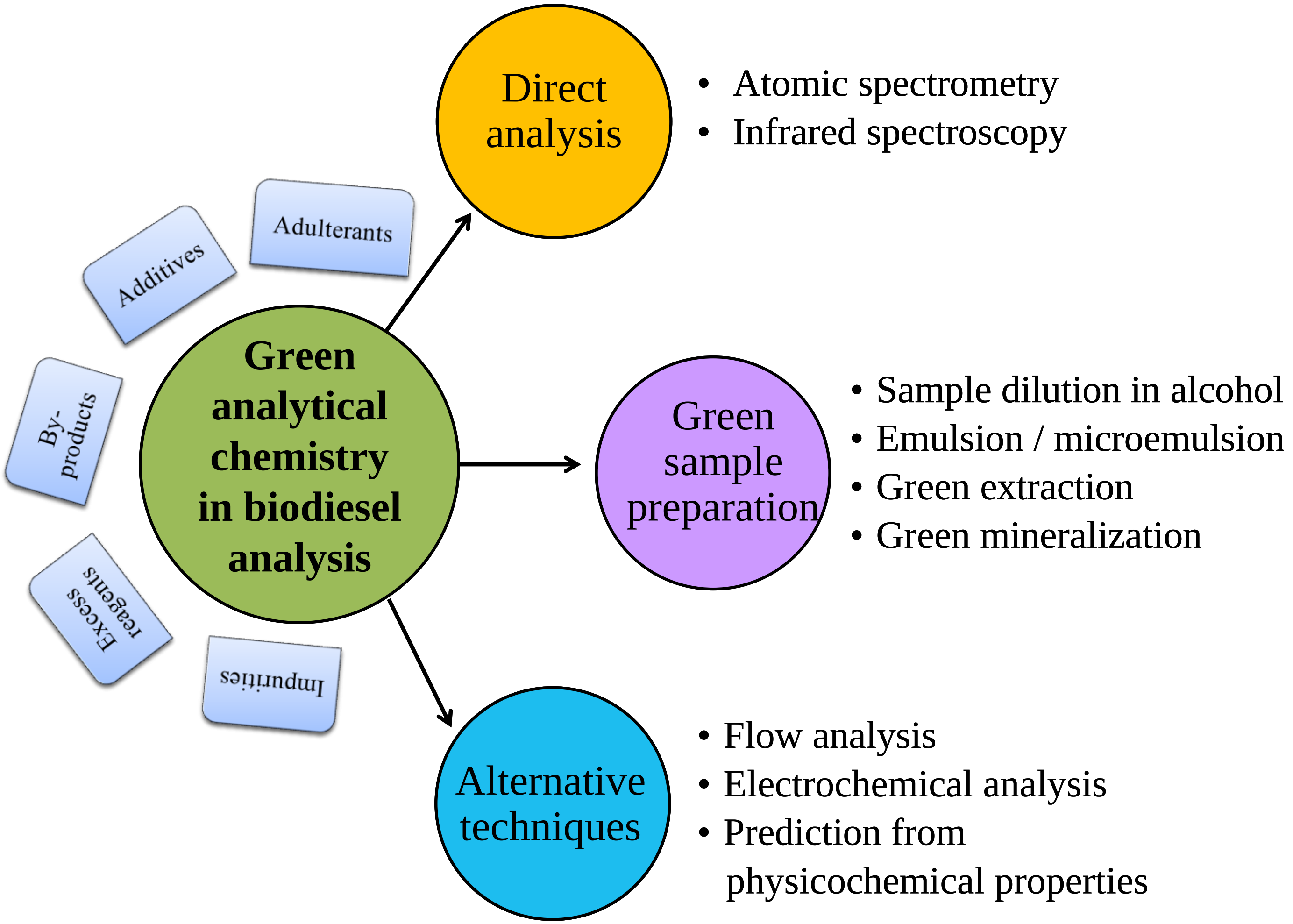
Direct analysis
• Atomic spectrometry
• Infrared spectroscopy
Adulterants
Additives
By-products
Excess reagents
Impurities
Green analytical chemistry in biodiesel analysis
Green sample preparation
• Sample dilution in alcohol
• Emulsion / microemulsion
• Green extraction
• Green mineralization
Alternative techniques
• Flow analysis
• Electrochemical analysis
• Prediction from physicochemical properties

Figure 1

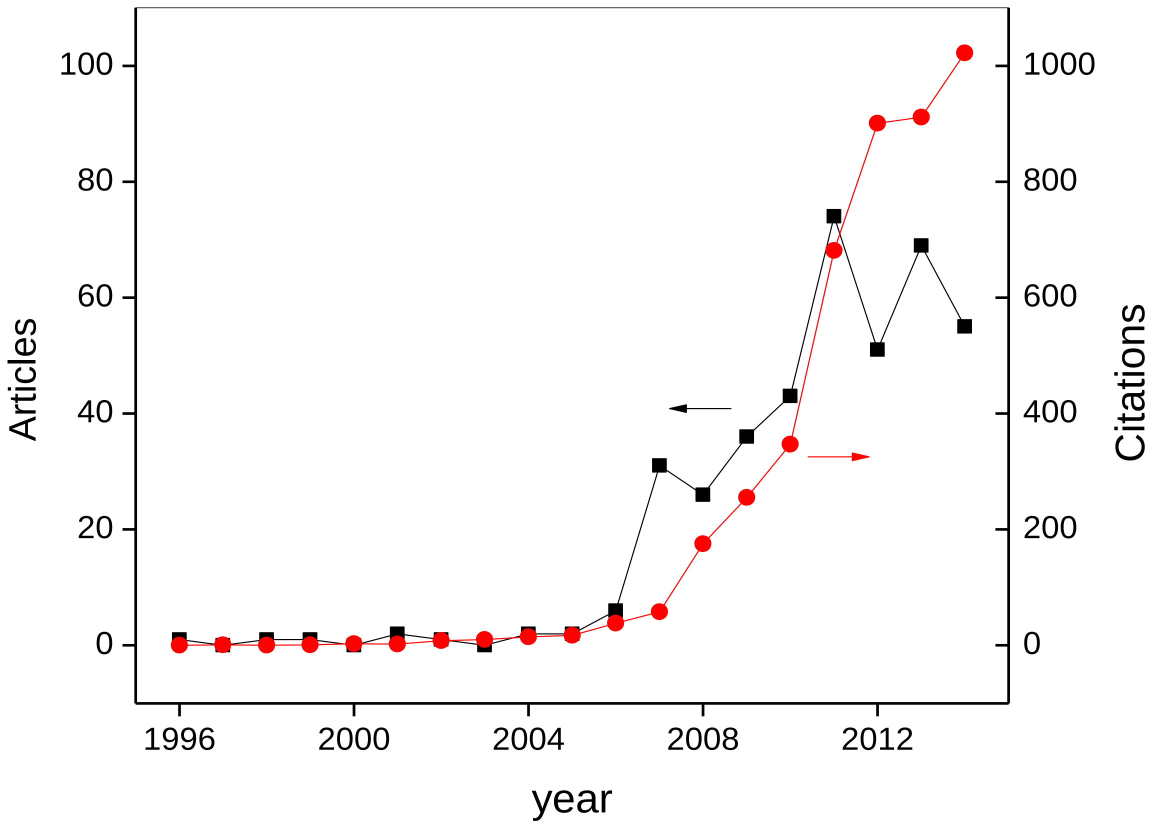
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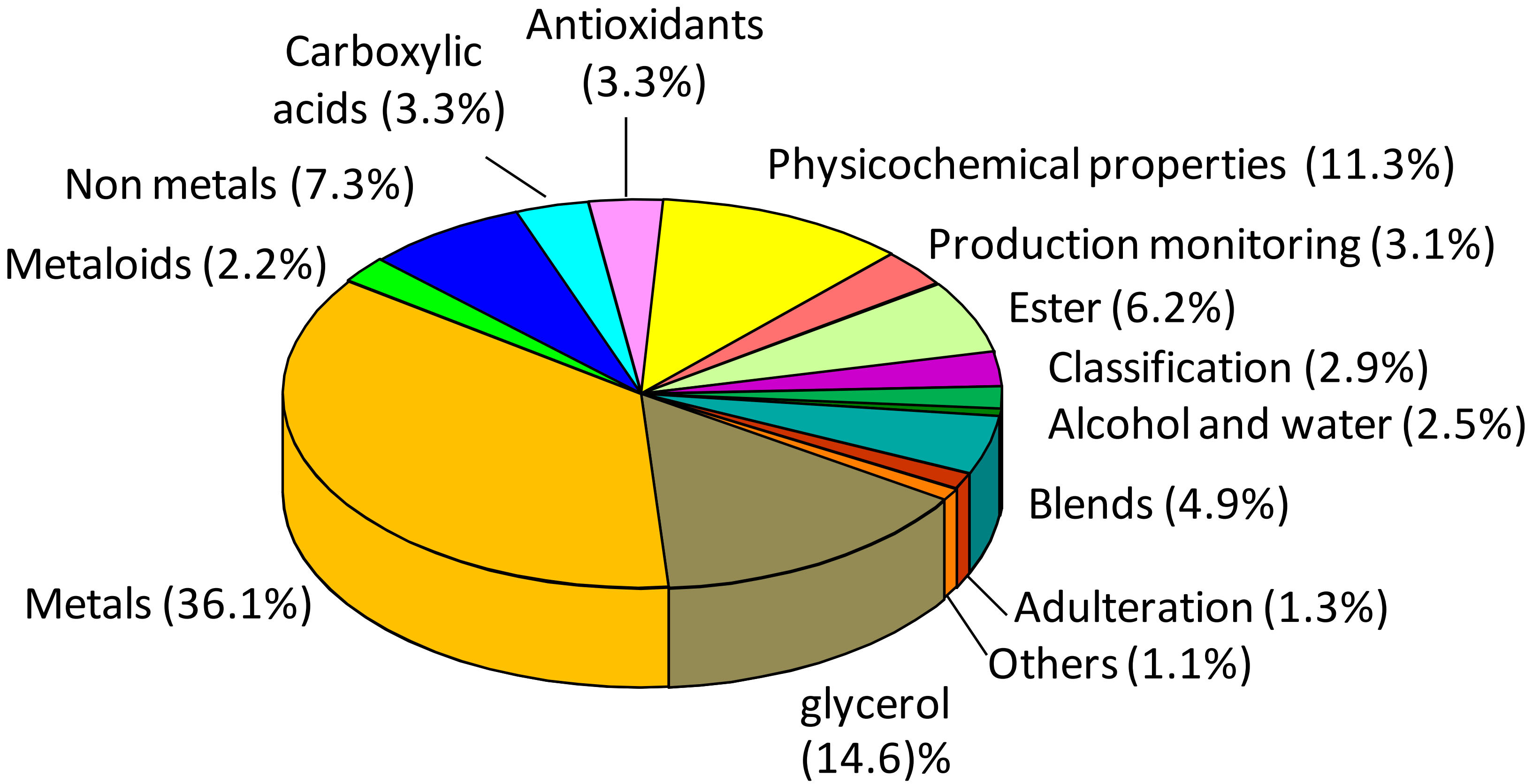
Figure 2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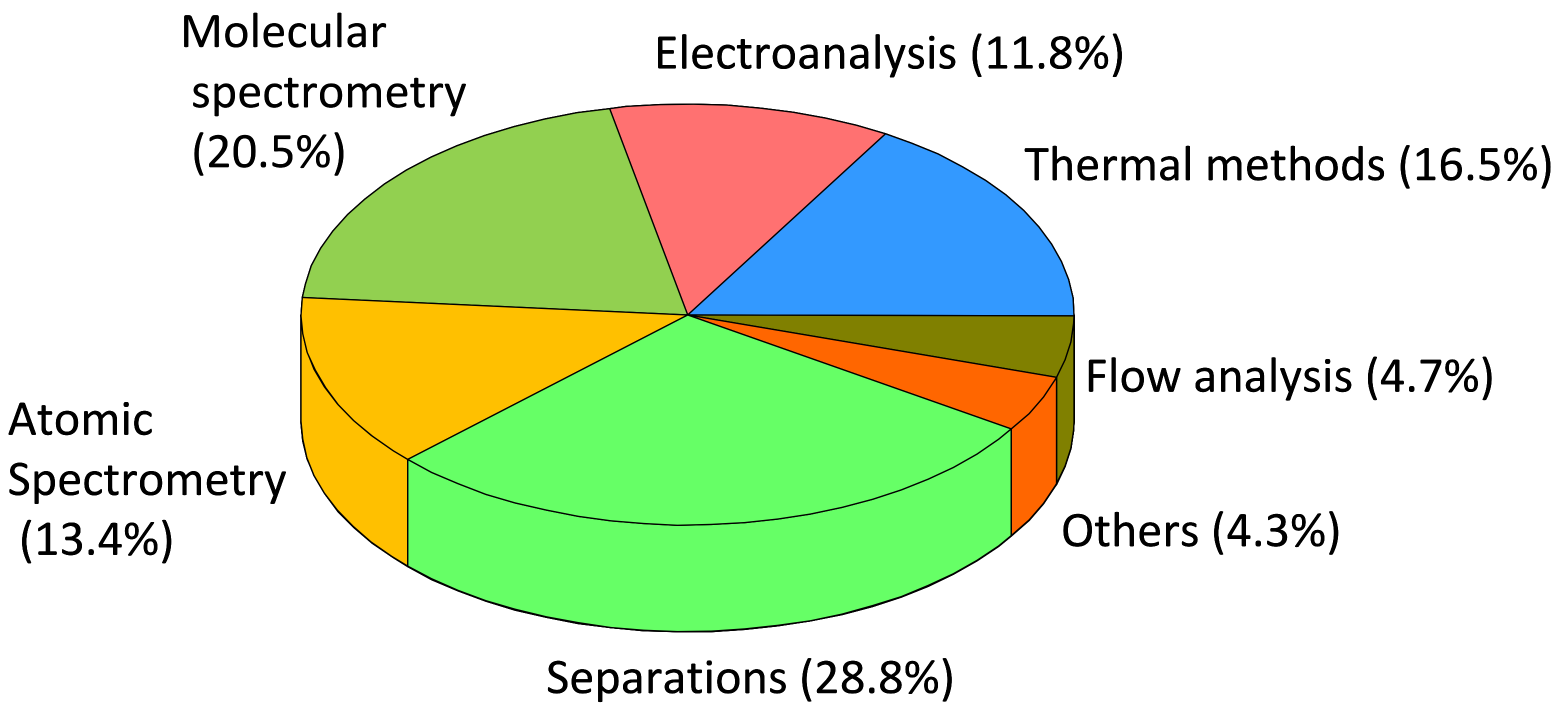
Carboxylic acids (3.3%)
Physicochemical properties (11.3%)
Non metals (7.3%)
Production monitoring (3.1%)
Metaloids (2.2%)
Ester (6.2%)
Classification (2.9%)
Alcohol and water (2.5%)
Blends (4.9%)
Metals (36.1%)
Adulteration (1.3%)
Others (1.1%)
glycerol (14.6)%

Figure 3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Electroanalysis (11.8%)
Thermal methods (16.5%)
Flow analysis (4.7%)
Others (4.3%)
Atomic Spectrometry (13.4%)
Separations (28.8%)

Figure 4