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Article

Optimization and Validation of FTIR Method with Tetrachloroethylene for Determination of Oils and Grease in Water Matrices

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O desenvolvimento de metodologias para a determinação de óleos e gordura (OG) apresenta inúmeras dificuldades e desafios para laboratórios analíticos, principalmente na análise de água potável devido ao seu baixo valor paramétrico. O método de FTIR após extração com tetracloroetileno foi desenvolvido para o monitoramento e controle de OG em vários tipos de água, tais como águas naturais (água superficial, água subterrânea e água de banho), efluentes e água potável. O método foi validado após aplicação de testes estatísticos e cálculo da estimativa de incertezas, e provou ser uma boa alternativa ao Freon 113. Os resultados referentes aos estudos de linearidade, recuperação, precisão, limites de detecção, limites de quantificação e estimativa de incerteza são apresentados e discutidos. O método validado foi aplicado às várias matrizes de água e demonstrou ser adequado ao monitoramento de óleos e gordura nestas matrizes em concentrações maiores que 0,01 mg L^{-1}.

The development of methods for oils and grease (OG) determination presents several difficulties and challenges for analytical laboratories, mainly for drinking water analysis regarding its low parametric value. FTIR method with tetrachloroethylene was developed for OG monitoring and control in several water matrices, such as natural waters (surface water, groundwater and bathing water), wastewaters and drinking water. The method was validated through the application of statistical tests and calculus of the uncertainty of the analytical assay, and proved to be a good alternative to Freon 113. Results regarding the calibration, linearity, OG recovery, precision, limits of detection and quantification, and uncertainty estimation are presented and discussed. The validated method was applied for the analysis of water samples from all studied matrices and was found to be appropriate for monitoring oil and grease in these matrices at levels higher than 0.01 mg L^{-1}.

Keywords: oils and grease, FTIR, water analysis, tetrachloroethylene

Introduction

It is clear that nowadays and in the near future, our society will have to face serious problems, such as over-exploitation of resources, environmental degradation, deterioration of ecological goods and services, for sustainable development. Among them, the availability of water of good quality is a critical issue since it represents an essential component for sustainable socio-economic development.

Urban wastewaters, which include domestic and some industrial waters, among others, have a strong contaminating effect on the natural aquatic systems, even when they are submitted to treatment because multiple organic compounds, escape conventional wastewater treatments and some of them may become ubiquitous in the environment and sometimes more toxic. This impact varies according to the treatment applied and chemicals present in wastewater.

The concentration of dispersed oils and grease (OG) is an important parameter for water quality and safety. OG in water can cause surface films and shoreline deposits leading

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to environmental degradation, and can induce human health risks when discharged in surface or ground waters.

OG is a measure of a variety of substances including fuels, motor oil, lubricating oil, hydraulic oil, cooking oil and animal-derived fats. Although not particularly toxic in and of itself, OG is a key indicator of water pollution and has many deleterious effects in the environment. In sewage, it hinders the proper operation of the water treatment facility. In storm drains, it makes an efficient cover, preventing aeration and providing a food source for bacteria leading to anaerobic conditions.[1-3]

Total petroleum hydrocarbon (TPH) is a term used to describe a broad family of several hundred chemical compounds that originally come from crude oil. In this sense, TPH is really a mixture of chemicals. They are called hydrocarbons because almost all of them are made entirely from hydrogen and carbon. The term TPH was traditionally used to classify aliphatic hydrocarbon materials. Because modern society uses so many petroleum-based products (for example, gasoline, kerosene, fuel oil, mineral oil and asphalt), the contamination of the environment by them is potentially widespread. Contamination caused by petroleum products will contain a variety of these hydrocarbons. Because there are so many, it is not usually practical to measure each one individually. However, it is useful to measure the total amount of all hydrocarbons found together in a particular sample of soil, water or air.[4]

Although OG is the conventional term used to define pollutants of this nature, including TPH, there are other terms that include the used extractant, like *n*-hexane extractable materials (HEM) and silica gel treated *n*-hexane extractable material (SGT-HEM). The term HEM indicates the extractant solvent (hexane) and that method may be applied to other materials than oil and greases. SGT-HEM indicates that the method may be applied to other materials than aliphatic petroleum hydrocarbons that are not adsorbed by silica gel.[5]

Sources of oil and grease are mainly anthropogenic. Oil and greases need to be contained and/or recycled typically to keep them from entering the environment. Domestic cooking oil should be poured into a disposable container and thrown out in the trash. Used motor oil and hydraulic fluids should be disposed of at a local automotive part store or a certified hazardous waste facility. Spill prevention kits should be used to help to clean up spills that occur at the work place.[2]

Since the oil and grease constituents and petroleum hydrocarbons are defined based on results from the test procedure, interferences are precluded by definition. However, the interpretation of test results on the basis of chemical structure, pollution potential or treatability should be approached with caution because of the diversity of substances measured by this procedure.

Organic solvents and certain other organic compounds not considered as OG on the basis of chemical structure may be extracted and measured as OG. From those measured, certain ones may be adsorbed by silica gel while others may not. Those which are not adsorbed are measured as petroleum hydrocarbons.[6]

Whereas to enable water-supply undertakings to meet the quality standards for drinking water, appropriate water-protection measurements should be applied to ensure that surface and groundwater are kept clean. Whereas the same goal can be achieved by appropriate water-treatment measurements to be applied before supplying.[7]

The production of safe drinking water is an important issue and the legislation establishes the levels of chemical substances allowed in drinking water, whether occurring naturally, as deliberate additions or as contaminants. As a consequence of European Union legislation,[7] and their recent implementation for national law,[8] the number of organic compounds in drinking water to be monitored is higher but there is no parametric value related to OG, or even TPH.

However, the national law,[9] conforming to European Directive 75/440/CEE,[10] sets a limit of 0.2 mg L^{-1} for surface waters intended for human consumption after conventional treatment. On the other hand, because OG can cause serious problems in the operation of wastewater treatment plants, the same law,[9] and the requirements of directive 76/464/CEE,[11] has set a limit of 15 mg L^{-1} of total OG for industrial discharges in the sewer system.

Oil and grease analyses, like many analytical methods for determination of water quality, do not measure a specific substance or compound. Oil and grease analyses attempt to quantify compounds which have a greater solubility in an organic solvent than in water, therefore OG and TPH include any material that may be recovered as a substance that is soluble in the solvent extractant. These include substances such as relatively non-volatile hydrocarbons, vegetable oils, animal fats, fatty acids, waxes, soaps, greases and related materials. The contribution of each of these substances will depend upon the origin of the wastewater being analyzed and the type of extracting solvent used. While imposing relatively simple analytical requirements, OG tests result in measurement of a broad range of compounds with widely varying chemistry and toxicity. Several methods are available for identification of specific organic fractions but tend to be too demanding of expertise, time and equipment to be used as a regulatory tool.[12]

OG in water is commonly determined by extraction into a non-polar, hydrocarbon free solvent followed by a gravimetric method or measurement of the infrared absorption spectrum of the extract.

For liquid samples, the 21st edition of Standard Methods for the Examination of Water and Wastewater[13] specifies four alternative methods for OG determination: the liquid-liquid partition-gravimetric method (5520 B), the partition-infrared method (5520 C), the Soxhlet method (5520 D), and the solid-phase, partition-gravimetric method (5520 G). Method 5520 C is designed for samples that might contain volatile hydrocarbons that otherwise would be lost in the solvent-removal operations of the gravimetric procedure. Method 5520 D is the method of choice when relatively polar, heavy petroleum fractions are present, or when the levels of nonvolatile greases may challenge the solubility limit of the solvent. For low levels of OG (< 10 mg L^{-1}), method 5520 C is the method of choice because gravimetric methods do not provide the needed sensitivity. Method 5520 F can be used in conjunction with other methods (5520 B, 5520 C, 5520 D or 5520 G) to obtain a hydrocarbon measurement in addition to, or instead of, the OG measurement. This method makes use of silica gel to separate petroleum hydrocarbons from the total OG on the basis of polarity.

Throughout the various editions of the Standard methods, different extraction solvents were used such as hexane and petroleum ether (12th edition) and Freon 113 (13th to 17th edition). However, because of environmental problems associated with chlorofluorocarbons, an alternative solvent (80% *n*-hexane and 20% methyl-*tert*-butyl ether) was included for gravimetric methods in the 19th edition. In the 20th and 21st edition, Freon 113 was dropped from all gravimetric procedures (retained for 5520 C), and replaced with *n*-hexane. In detailed studies involving wastewaters and solid matrices, it was shown that *n*-hexane produced results statistically different from results produced by Freon 113.[13-15] Thus, method 5220 B defines OG as any material recovered as a substance by Freon 113 from an acidified sample.

EPA Method 1664 A requires use of *n*-hexane as the extraction solvent. However, there may be samples for which Method 1664 may not be suitable, e.g., when it is desirable to measure *n*-hexane or other components with a boiling range near or below the boiling point of *n*-hexane. In those situations, analysts in the past may have used EPA Method 418.1 "Petroleum Hydrocarbons, Total Recoverable" for determining TPH in water. However, this method uses Freon 113. EPA specifies method 1664 as "*n*-Hexane Extractable Material (HEM) and Silica Gel Treated *n*-Hexane Extractable Material (SGTHEM) by Extraction and Gravimetry". The silica gel treatment allows for the separate determination of total petroleum hydrocarbons (TPH).[14]

Although gravimetric methods[5,13] are simple, quick and inexpensive, they present the disadvantages of low sensitivity (usually limits of detection are 5-10 mg L^{-1}), loss of constituents that volatilize at temperatures above those used for the evaporation of the solvent and inclusion of compounds which are not oil and grease but are extracted by the solvent and therefore contribute to the final mass.

Infrared-based methods are generally more sensitive.[16] There are several standard test protocols based around this methodology with many solvents, like tetracloromethane (the first solvent used in this method), *n*-hexane and 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113), most commonly using Freon 113.

IR methods measure the absorbance of the C–H bond, i.e., the stretching of aliphatic CH_2 groups at 2930 cm^{-1}, of CH_3 groups at 2960 cm^{-1} and of aromatic C–H bonds at 3010-3100 cm^{-1}. It is self-evident that IR methods can only use solvents without C–H bonds, but since no evaporation of the solvent is needed, there are no losses of volatile components.[16] Three well-known IR methods for OG determinations are SMWW 5520 C,[13] EPA 418.1[6] and ASTM D 3921-96.[6] All these methods use Freon 113 as the extraction solvent.

The 1987 Montreal Protocol on Substances that Deplete the Ozone Layer is a landmark agreement that has successfully reduced the global production, consumption and emissions of ozone-depleting substances (ODSs). Since this protocol and its amendments (1990 and 1992) settled a timetable for the phase-out of the production and consumption of some solvents, manufacture and their use have dropped and some of them became illegal. As example, the use of Freon 113 was phased out by 1996 and the use of tetrachloromethane became illegal in 2010.

These facts have resulted in the development of new official methods for OG analyses that use hexane and more recently the use of tetrachloroethylene.

This study began as a result of lack of information on the use of tetrachlorethylene (solvent extraction) in the analysis of OG by FTIR method in different water matrices, according to the requirements of national and international legislation, especially studies that allow accreditation of the method. In natural waters and wastewaters, the quantification of any target compound with a guidevalue in the order of ng L^{-1} and the accreditation of the method for their quantification, requires compliance with all validation requirements for each matrix under study.

Tetrachloroethylene (C_2Cl_4) is a rather non-volatile (b.p. 121 °C), nonhydrocarbon solvent with infrared transparency that makes it suitable for IR measurements in the region of C–H bond absorption.

Farmaki *et al.*[16] validated an FTIR method for the determination of OG in water with this solvent, and also the ASTM standard test use this solvent,[6] but there are some limitations regarding the analysis of drinking water.

The study of Farmaki *et al.*[16] is the only work to date with the use of tetrachlorethylene as extraction solvent on water analysis but cannot respond to current legislative requirements for water for human consumption. The method validated by the group has limits of detection (LOD) and quantification (LOQ) of 0.1 and 0.3 mg L^{-1}, respectively. LOQ is higher than the guideline value of 0.2 mg L^{-1} for surface waters intended for human consumption after conventional treatment and LOD is higher than the requirements of national law (0.01 mg L^{-1}).[9] The validated method was only tested in recovery assays of tap water and surface water (lake), but was not used in routine analysis. Moreover, in the accreditation of an analytical method is necessary to calculate uncertainties, but it was not performed in this study.

The liquid-liquid extraction (LLE) is a useful technique, but it is laborious, time consuming and requires large amounts of frequently toxic organic solvents and for these reasons, it has been developed other extraction methods, such as microwave extraction methods[17] and solid phase extraction methods (SPE).[18-22] But there are some sorbents that have limited selectivity and, in addition to OG, many matrix constituents can also be enriched and disturb the FTIR spectra. The SPE method without elution step with organic solvent[22] also has some time limitations because the water must be removed as far as possible from the disk and several spectra must be recorded in several position of the filter. However, its selectivity can become a major drawback in the analysis of wastewater where the objective is to analyze the total OG from an aqueous matrix. Moreover, in case of wastewaters, because they have a lot of material in suspension, disk or filter may be clogged or blocked. This problem is overcome in LLE with a good selection of solvent extraction.

For the analysis of TPH, other methods have been used such as gas chromatography with a flame ionization detector (GC-FID)[23] and gas chromatography-mass spectrometry (GC-MS),[19,24] which have the advantage of providing identification or even fingerprinting capabilities, but it is often difficult to extrapolate their results in order to obtain the total OG values.[16] Knowledge of the quantity of OG present is helpful in proper design and operation of wastewater treatment systems and also may call attention to certain treatment difficulties.[13]

To meet the requirements of national and community legislation on OG, laboratories must use IR methods, which are more sensitive than gravimetric methods, but less specific than chromatographic methods. Therefore, it is necessary to implement methods of extraction with other solvents, as a substitute to Freon 113 and validate the modified methods.

The accredited laboratories must demonstrate in accreditation audit that validated method meets the requirements of International Standard Organization, Eurachem guides for calculating uncertainties and recoveries studies in all target matrices under accreditation. The freon replacement for tetrachlorethylene created serious problems for laboratories because the replacement of solvents with different characteristics cannot show similar results. This problem is greater in water for human consumption, which guidevalue is lower.

The aim of this study is to present the development and validation data of an FTIR method for determination of OG in waters using tetrachloroethylene, an alternative solvent that has already been proposed in ASTMD 3921-96,[6] as the extraction solvent. Tetrachloroethylene does not have the adverse environmental effects of Freon 113 and it is well-suited to OG determination because it is an excellent solvent for organic compounds and has no C–H groups. This in-house method was developed in the Laboratório de Águas do Litoral Alentejano (LALA, Sines, Portugal) in order to be applied for monitoring OG in several water matrices, like natural waters (surface water, groundwater and bathing water), wastewaters and drinking water supply network.

The LALA laboratory is located in a heavily industrialized area, which has an environmental monitoring program controlled and supervised by the competent authorities, ensuring that the limits imposed by legislation are met. The LALA laboratory is an accredited laboratory that uses FTIR with Freon 113 to measure OG. Changing the solvent extraction required the laboratory to validate the method with the new solvent and undergo re-evaluation by the competent authorities for accreditation.

The FTIR method with tetrachloroethylene was validated through the application of several statistical methods which allow defining the linearity, working ranges, instrumental analytical limits (limits of detection and quantification), and the limit of determination of the global method. Simultaneously, the precision and the matrix effect of the analytical assay was studied, according to the requirements of Council Directive 98/83/EC[6] and international procedures.[25] An evaluation of the main uncertainty sources of this method was also included, which allowed an estimative of the expanded uncertainty for the OG determination. Method accuracy was also assessed by inter-laboratory assay.

The method accreditation is renewed annually and it was renewed with the tetrachloroethylene as extraction solvent.

Experimental

Instrumentation

The OG determination was performed using an infrared spectrometer, a FTIR instrument operating in single mode, Perkin Elmer PE 1600 FTIR (Perkin-Elmer, Waltham, MA, USA) equipped with rectangular quartz cells of 10 mm path length and cylindrical quartz gas cells of 50 and 100 mm path length with 25 mm diameter and two PTFE stoppers.

The following materials were used: filter paper, ashless, quantitative, general-purpose, 11 cm or equivalent (Whatman, Kent, England), glass bottle, approximately 1.0 L, with screw cap having a TFE-fluorocarbon liner, graduated cylinder of 1.0 and 2.0 L, separatory funnel, 2.0 L, with TFE-fluorocarbon stopcock (one for each sample analyzed).

The stirring of aqueous solutions was performed using a hot/stirring plate, DataPlate® Digital from Biomolecular Inc. (Reno, NV, USA) with small TFE-fluorocarbon stirring bar (Bibby Sterilin, Staffordshire, UK).

Reagents and standard solutions

All aqueous solutions and serial dilutions were prepared by dilution with ultra-pure water (Mili-Q Biocel System, Millipore, Billerica, MA, USA).

The standard oil (stock solution) was prepared by mixing 37.5% v v^{-1} isooctane (2,2,4-trimethylpentane), 37.5% v v^{-1} hexadecane and 25.0% v v^{-1} benzene. All reagents were pro-analysis grade and were supplied by Merck (Darmstadt, Germany). The stock solution and serial dilutions were prepared with ultra-pure water.

Sodium sulfate (Na_2SO_4) anhydrous, concentrated sulfuric acid and silica gel 6 (100 to 200 mesh) were pro-analysis grade and were supplied by Merck. The Freon 113 (1,1,2-trichloro-1, 2,2-trifluoroethane, pro-analysis grade) was supplied by VWR (BDW Prolabo®, Fontenay-Sous-Bois, France). The tetrachloroethylene (pro-analysis grade) was supplied by VWR and by JT Baker (Deventer, Netherlands).

FTIR conditions and calibration

The background was established with extraction solvent. A single beam scan was made for each cell (10, 50 and 100 mm) with extraction solvent over the range 3200-2700 cm^{-1} at 8 cm^{-1} resolution with 35 s acquisition time and accumulating 16 scans *per* sample and standard. Each of the standards and samples was scanned, and the peak maximum at 2930 cm^{-1} less the baseline from 3200 to 2700 cm^{-1} was used in the linear regression.

If the absorbance exceeded 0.8 for a sample, a shorter path length was selected or the sample was diluted as required. Standards were used to prepare a calibration curve.

Before processing any standards, a blank of solvent was analyzed. Before processing any samples, a blank of ultra-pure water treated in the same way as the samples were analyzed.

Eventual cell residues from previous analysis will cause inaccuracies, low sensitivity and lack of precision. The cells were only cleaned with lens cleaning paper to wipe the optical surfaces because most paper products contain wood fibers which may scratch or damage the cell face or surface. At the end of the day, the cells are well cleaned and stored in their box after drying. Between standards and samples, the cells were washed with solvent. Weekly or when the blanks were not appropriated, the cells were rinsed with solvent, warm water with detergent, diluted acid solution (2 mol L^{-1} HCl) and with ultra-pure water.

Validation studies

A laboratory applying a specific method should have documented evidence that the method has been appropriately validated. This holds for methods developed in-house, as well as for standard methods. The purpose of the validation process is to provide a high degree of assurance that a specific process will consistently produce a product (control information or data) which meets predetermined specifications and quality attributes.

The main objective and one of the main concerns are the fulfillment of the requirements or acceptance criteria of the national or international standards or regulations, and actions in case of deviations.

The validation parameters that were examined, based on the aforementioned protocol, were linearity, limits of detection and quantification, repeatability, reproducibility, accuracy, recovery, and uncertainty.

Linearity was studied for three concentration ranges and quartz cells with three different path lengths: low range with 100 mm cells, middle range with 50 mm cells and high range with 10 mm cells. Six solutions containing OG at different concentration levels (0.15-3.8, 3.8-75 and 75-375 mg L^{-1}) were measured against pure solvent tetrachloethylene as background spectra. The calibration curve was plotted applying least-square regression analysis. Linearity was evaluated by the values of determination coefficients and coefficient of variation of the method, and after application of Mandel test. The results were treated in order to comply with all the defined limits for each statistical test. The calibration curves were repeated monthly for evaluation of their precision by the analysis of the relative standard deviation (RSD) of the slope. The calibration curves with Freon 113 and tetrachroethylene were compared by correlation analysis of absorbance values obtained with both solvents.

Ten standard samples of each of the lowest (x_1) and the highest (X_N) concentrations for each linear range were analyzed separately. A total of 2 × n (n = 10) measurements (y_i) was obtained from each series. The means, y_1 and y_N, and relative standard deviation, RSD_1 and RSD_N, were calculated for both datasets.

These solutions were analyzed to evaluate the repeatability of the FTIR method. The values of LOD and LOQ of the FTIR method were calculated using the repeatability data and the formula (LOD = 3 × standard deviation) and (LOQ = 10 × standard deviation), respectively, or using the calibration line method (LOD = 3 × relative standard deviation/slope and LOQ = 10 × relative standard deviation/slope).[25]

LOD and LOQ of the LLE-FTIR method (global method) were calculated for the studied matrices (distributed by each range and quartz cells) at one concentration level. Ten samples of each matrix were spiked with 0.01, 0.3 and 2 mg L^{-1} of OG corresponding to the low range (drinking water, groundwater and surface water), middle range (bathing water and some wastewaters) and high range (mainly, wastewater), respectively. LOD and LOQ were also calculated using the formula (3 × SD) and (10 × SD), respectively. Homogeneity of variance analysis was applied to the best and the worst standard deviation data from these three matrices.

The intra-laboratory reproducibility of the FTIR method was also evaluated after analysis of two concentration levels of the linear range, in different days.

The repeatability and intra-laboratory reproducibility of the global method were studied in all matrices at two concentration levels. The repeatability and intra-laboratory reproducibility of the method were expressed as a relative standard deviation, RSD_r and RSD_R, respectively.

Any analytical method involving an extraction step is at risk of negative bias introduced by incomplete extraction and any analytical method applied to the different matrices can be influenced by different interferences. In addition to the findings above, recovery studies were performed in two series for each of the different sources of water (drinking water, natural waters and wastewaters) and at several concentrations from 0.01 (low range) to 2 mg L^{-1} (high range). The measurements were carried out against unspiked water from the same source.

Analysis of variance (ANOVA) was used to evaluate the analysis data (repeatability, reproducibility and recovery), and significant differences among means were determined by one-way analysis of variance.

The main uncertainty sources of the method were identified and quantified, followed by the determination of the combined standard uncertainty (u_c) using a Gauss propagation model. The last step for uncertainty evaluation of an analytical result was the calculation of the expanded uncertainty (U), using a coverage factor k = 2 (95% of confidence level). Two of the approaches to calculate uncertainty are the bottom-up and the top-down methods. The bottom-up method was proposed by ISO (International Organization for Standardization) in order to quantify uncertainty in physical measurements and was subsequently adopted by Eurachem. On the other hand, the top-down method uses validation data and data from proficiency test schemes to estimate the uncertainty of the method.[26-28]

Using the bottom-up approach, we combined the components of uncertainty related with the calibration curve (u_{curve}), the standard solution preparation ($u_{standard}$), and the intermediate precision of the method ($u_{precision}$). The standard uncertainty of the standard solution preparation is the combination of other components of uncertainty like the purity of the commercial standard, the volumetric and the weight measurements. The combined uncertainty (u_c) was calculated using the following equation:

$$u_c = \sqrt{u_{precision}^2 + u_{standard}^2 + u_{curve}^2} \quad (1)$$

Using the 'top-down' method we combined the components of uncertainty related with the variability (u_{var}) and with the accuracy ($u_{standard}$) of the recovery studies (matrix effect). The uncertainty related with the variability (u_{var}) combined the components of uncertainty related with the intermediate precision of the standard control of lowest concentration ($u_{standard}$) and intermediate precision of the duplicate samples (u_{dup}). The variability uncertainty (u_{var}) was calculated using the following equation:

$$u_{var} = \sqrt{u_{standard}^2} + \sqrt{u_{dup}^2} \quad (2)$$

In this approach the combined uncertainty (u_c) was calculated using the equation:

$$u_c = \sqrt{u_{var}^2 + u_{accuracy}^2} \quad (3)$$

The expanded uncertainty (U) was obtained by multiplying u_c by a coverage factor (k) using the following equation:

$$U = k \times u_c \quad (4)$$

In general, the value of the coverage factor *k* is chosen on the basis of the desired level of confidence to be associated with the interval defined by u_c. Typically, *k* is in the range 2 to 3. When the normal distribution applies and u_c is a reliable estimate of the standard deviation, $U = 2\ u_c$ (i.e., $k = 2$) defines an interval having a level of confidence of approximately 95%, and $U = 3\ u_c$ (i.e., $k = 3$) defines an interval having a level of confidence greater than 99%.

The last approach was considered for estimation of the expanded uncertainty of the analytical method.

The method accuracy was assessed by inter-laboratory assay promoted by Associação de Laboratórios Acreditados (RELACRE, Portugal) concerning wastewater analysis (Reference ECI/AR/1-2011). The assigned value and the satisfactory range for OG in wastewater was 23 ± 3 mg L^{-1}.[29]

Method accuracy was also determined by the analysis of two standard controls for each linear range.

Water samples

The sampling step was according to the requirements of standard methods[13] and ASTM D3921-96.[6] The sample was collected using a glass amber bottle equipped with a screw cap having a TFE-fluorocarbon liner. The sample containers were not overfilled and the samples were not subdivided in the laboratory. Replicate samples were collect for replicate analysis.

Typically, a sample of about 0.5, 1.0 or 2.0 L was required for the assay of wastewaters, natural waters (surface water, groundwater and bathing water) and drinking waters, respectively.

The samples were stored at 5 ± 3 ºC until analysis. The entire sample was used, i.e., no portion was removed for other tests. The sample was preserved with a sufficient quantity of either sulfuric acid 1 + 1 v v^{-1} to attain a pH 2 or lower. The amount of reagent required was dependent on the pH of the sample at the time of collection and upon its buffer capacity.

In 2011, 841 samples were collected from several sampling points of the main water sources analyzed by our laboratory and the optimized and validated LLE-FTIR method was applied to analyze these samples.

After carefully transferring the sample to a separatory funnel, the sample bottle was rinsed with 30 mL of solvent extraction. This solvent was used in the first extraction of the sample and two more successive extractions of the sample were made with 30 mL of extraction solvent (Freon 113 or tetrachloroethylene). The extracts were filtered through a funnel containing a filter paper with 10 g of Na_2SO_4, both of which were solvent-rinsed with an additional 10 to 20 mL solvent, into a clean 100 mL volumetric flask. The extracts were combined and diluted to 100 mL with the extraction solvent.

Some wastewaters may contain high amounts of OG. For these samples, it was necessary to use a 100 mL sample rather than 500 mL or to dilute the sample before the extraction.

For the analysis of total petroleum hydrocarbons, 1.5 g of silica gel was added to the 50 mL of extract and stirred for 5 min and the extract was filtered through a funnel containing a filter paper moistened with solvent. Sufficient extract to fill a quartz cell was withdrawn and a second scan was made of the silica-gel-treated extract for the determination of the petroleum hydrocarbons concentration.

Results and Discussion

Linearity

The OG calibration was performed by external standard calibration. The linear ranges, the determination coefficient (r^2), the coefficient of variation of the method (CV_m), the residual analysis and the parameters of Mandel test (the PG-values and the *F*-value of Fisher/Snedecor) are given in Table 1.

Table 1. Regression data for OG by FTIR method

Calibration curve	Low range (N = 6)	Middle range (N = 9)	High range (N = 6)
Quartz cells / mm	100	50	10
Linear range / (mg L^{-1})	0.15-3.8	1.5-75	75-375
Slope (b)	0.0195	0.0104	0.0017
Intercept (a)	0.0088	0.0046	0.0096
r^2	0.9987	0.9980	0.9989
CV_m / %	5.2	3.4	2.2
PG ≤ F (1; N-3; 95%)	−3.0 < 10.1	−6.0 < 5.99	−3.0 < 10.1

N: number of data points; F: value of Fisher/Snedecor (tabulated value); r^2: determination coefficient; CV_m: coefficient of variation of the method.

All calibration curves fulfilled all the requirements for a linear method with determination coefficients higher than 0.995, coefficient of variation lower or equal to 6.0% and PG-values lower than the tabulated value of the Fisher/Snedecor.

The calibration curves in all ranges were precise. The relative standard deviation of the slopes from the six calibration curves showed RSD of 3.5% ($0.0188 \leq b \leq 0.0201$), 4.3% ($0.0093 \leq b \leq 0.0104$) and 2.4% ($0.0016 \leq b \leq 0.0017$) in low , middle and high ranges, respectively.

As our samples belong mainly to the high range, Figure 1 shows the FTIR spectra of oils and grease obtained for standard calibration of calibration curve in this range.

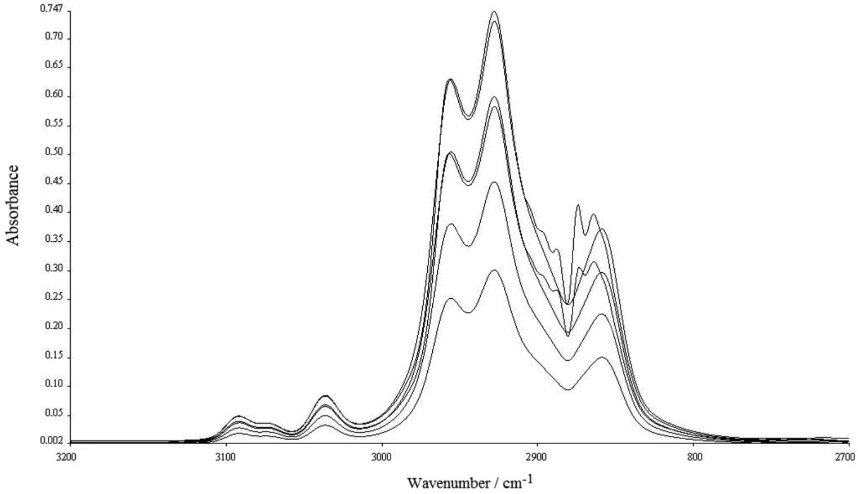

Figure 1. FTIR spectra of oils and grease obtained for calibration solutions.

The absorbance values of calibration standards with Freon 113 are plotted on a graph against the absorbance values of same calibration standard with tetrachloroethylene (standard absorbance with tetrachloroethylene *vs.* standard absorbance with Freon 113). Results are shown in Figure 2. There was a good correlation between both solvents (Freon 113 and tetrachloroethylene) with r^2 of 0.9910, 0.9984 and 0.9987 in low, middle and high ranges, respectively (N = 6).

Repeatability, intra-laboratory reproducibility and recovery

The repeatability and intra-laboratory reproducibility were studied by analyzing replicate samples of ultra-pure water spiked with different concentrations of OG according to the studied range (Table 2). These quality parameters are expressed as RSD. For the purpose of this method, it may be considered acceptable a RSD of 20% or less.[8,9]

Results in both conditions and for two concentration levels of each range showed a RSD lower than 8.0%, therefore, the FTIR method had a good precision.

The repeatability was also studied in real samples spiked with different concentration of OG according to the studied range. Table 3 shows the repeatability for OG by the LLE-FTIR method in real samples and the recovery results in these different matrices after fortification.

Results (Table 3) showed that there were no significant differences between the recoveries obtained for these matrices with values between 98 and 108%. The variability of the data was satisfactory with a RSD smaller than 15%.

Results in all samples and with different concentrations showed a RSD lower than 10%, therefore, the LLE-FTIR method had good precision.

As there were no significant differences between the recoveries obtained for the analyzed water matrices, an average value of the recovery data obtained in the analyzed water matrices (102%) was used to calculate the analytical limits of the global method (LLE-FTIR).

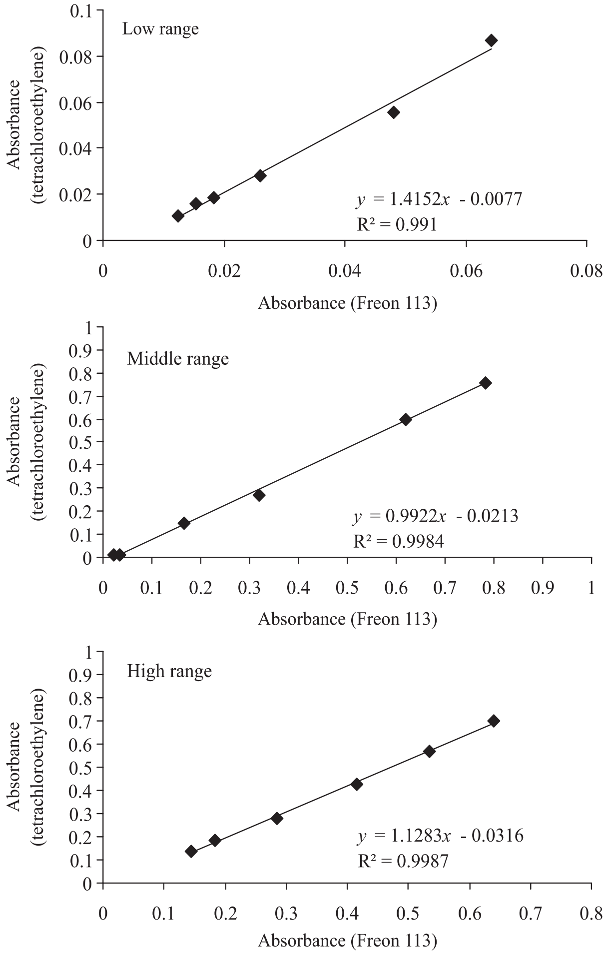

Figure 2. Calibration curves of OG (tetrachloroethylene *vs.* Freon 113) by FTIR method.

Table 2. Repeatability and intra-laboratory reproducibility for OG by FTIR method

	Low range		Middle range		High range	
Spiked level / (mg L^{-1})	0.15	3.8	1.5	75	75	375
Repeatability, RSD_r / %, n = 10	7.3	3.6	4.6	0.62	0.8	2.2
Intra-laboratory reproducibility						
RSD_R / %	6.3	5.3	3.3	4.7	5.7	5.9
n	102	53	73	73	153	146

RSD_r and RSD_R: repeatability and intra-laboratory reproducibility of the method expressed as relative standard deviation, respectively.

Table 3. Repeatability, recovery rates (R) and relative standard deviation (RSD) for OG by LLE-FTIR method in real samples

Matrix	Drinking water		Groundwater		Surface water		Bathing water		Wastewater			
Unspiked samples / (mg L^{-1}), n = 3	< 0.010		< 0.010		0.010		0.070		0.40		50	
Spiked level / (mg L^{-1})	0.012		0.010		0.010		0.012		0.34		2.0	
Recovery, n = 10	R / %	RSD / %	R / %	RSD / %	R / %	RSD / %	R / %	RSD / %	R / %	RSD / %	R / %	RSD / %
	102	11	102	6.2	100	3.5	101	3.1	108	5.0	98	2.5

Analytical limits

The values of LOD and LOQ of the instrumental method (FTIR) for low, middle, and high ranges calculated under repeatability conditions were 0.03 and 0.11, 0.51 and 1.7, and 1.4 and 4.9 mg L^{-1}, respectively. In high range the limit of quantification of the instrumental method (FTIR) is set to the first point of the calibration curve, 60 mg L^{-1}.

When 2 L of water samples (drinking water and natural waters) are extracted with 100 mL of extraction solvent (concentration factor of 20), the theoretical limit of quantification of the global method (LLE-FTIR) was 5.5×10^{-3} mg L^{-1} (instrumental LOQ/concentration factor). But with this concentration, the instrumental signal is too small and the recoveries did not fulfill the analytical requirements. For this reason, the limit of quantification of the global method (LLE-FTIR) was set at 0.01 mg L^{-1}.

For some wastewaters and bathing waters (mean range), LOQ of the LLE-FTIR method was 0.09 mg L^{-1} (for a concentration factor of 20).

The majority of the wastewaters analyzed in our laboratory belong to the high range. The extraction was made with 400 mL of water samples and 100 mL of tetrachloroethylene (concentration factor of 4) and the limit of quantification of the LLE-FTIR method was 15 mg L^{-1}.

So, the LLE-FTIR method showed different LOQ according to the values of OG in water samples and therefore the analytical range used.

These limits of quantification fulfilled the requirements of the European Council Directive 98/83/CE,[7] and national law,[9] and they were lower than values reported by other authors.[16]

Accuracy

The accuracy of the method was evaluated through the analysis of a wastewater test material (RELACRE, Reference ECI/AR/1-2011). OG content (24 mg L^{-1}) was close to the assigned value and within the reported range and the Z-score was 0.3.

The method accuracy was also determined by the analysis of two standard controls for each linear range as reported in Table 2. So, these standard controls were used to the study of precision and accuracy.

In low range, the relative errors were 5.5 (n = 56) and 4.8% (n = 53) for the standard control of the 0.15 and 3.8 mg L^{-1}, respectively. In middle range, the relative errors were 7.2 (n = 73) and 4.9% (n = 75) for the standard control of the 3.8 and 75 mg L^{-1}, respectively. In high range, the relative errors were 5.3 (n = 153) and 5.0% (n = 146) for the standard control of the 75 and 375 mg L^{-1}, respectively. Concerning the reported values the method showed a good accuracy.

Uncertainty study

One approach was considered for estimation of expanded uncertainty of the analytical method, top-bottom approach. Table 4 summarizes the results obtained.

The expanded uncertainty (U) was between 16 and 28%. The accuracy component of uncertainty had the highest contribution to the expanded uncertainty, in low and middle ranges.

Water samples

The validated LLE-FTIR method was successfully applied to the analysis of the presence of OG in 841 water

Table 4. Individual uncertainty components and respective expanded uncertainty (*U*) estimated in the determination of OG by LLE-FTIR method

Quality control		n	$u_{standard}$ / %	u_{dup} / %	$u_{accuracy}$ / %	*U* / %
Low range	Standard of 0.15 mg L⁻¹	90	5.7	1.9	5.5	26
	Recovery	36				
	Replicate analysis (n = 2)	35				
Middle range	Standard of 1.5 mg L⁻¹	44	2.0	2.9	4.8	16
	Recovery	17				
	Replicate analysis (n = 2)	12				
High range	Standard of 75 mg L⁻¹	100	5.9	5.4	2.1	28
	Recovery	10				
	Replicate analysis (n = 2)	60				

matrices receiving in the LALA laboratory, including 10 drinking waters, 209 natural waters (69 underground waters, 50 surface waters and 90 bathing waters), 179 industrial waters, 51 industrial effluents and 196 residual waters (influent and effluent) from wastewater treatment plant (WWTP). Results are presented in Table S1 in the Supplementary Information (SI) section.

The mean values and standard deviation of the concentration of OG in each water matrix cannot be presented because the values were not representative of a normal distribution. The distribution of positive samples is not symmetrical (bell-shaped) since most positive samples are concentrated near the LOQ values of the working range (some natural waters) or very far from the LOQ values. For this reason, we only show the median values.

In drinking water matrices, OG was not detected in all samples.

According to results presented in Table S1 in the SI section, there was an incidence of positive samples between 22 and 100% for groundwater and industrial water, respectively. The positive incidence was higher in industrial effluents and WWTP influents. The maximum detected values were 4.20×10^3 and 1.80×10^5 mg L⁻¹ in industrial effluents and WWTP influents, respectively.

Most groundwaters, bathing waters and surface waters did not show OG above LOQ with high incidence of negative samples.

Wastewater discharges has high concentrations of OG from industrial effluents and urban wastewater. In WWTP, the concentration of OG must be reduced before its elimination to receiving waters.

It is important to recognize that all municipal and industrial sewage, regardless of location, will contain OG and this issue is not unique to any particular municipal area. Each geographic area will differ only with respect to quantities and relative abundances of individual hydrocarbons.

The occurrence of OG in natural waters is a function of whether treatment technologies and types of treatment technologies employed for sewage. The LALA does not know which technologies were used, but after treatment there is a significant decrease of OG. The positive samples and the concentration levels of OG in WWTP effluent were reduced. The efficiency of elimination of OG was about 90% in WWTP but 21 water effluent samples (11%) had OG above 15 mg L⁻¹.[9]

The validated LLE-FTIR method was also applied to the analysis of TPH in 171 water matrices received, including 68 underground waters, 32 surface waters and 71 industrial effluents. Results are presented in Table S2 in the SI section.

Natural waters (groundwaters and surface waters) showed mostly a negative incidence of negative samples, as opposed to industrial effluents which had a positive incidence of 99%. In these samples, the maximum detected value was 3000 mg L⁻¹.

Groundwaters and industrial effluents showed 2 and 25 samples above the reference value, respectively.[9]

Conclusions

The LLE-FT/IR method was validated for the analysis of OG in water samples. Tetrachoroethylene was found to be a suitable replacement to Freon 113 for the determination of OG in waters. The method working range as well as its precision, accuracy and uncertainty are acceptable for the routine analysis of OG/TPH in several water matrixes, like drinking water, natural water (groundwater, surface water and bathing water) and residual water, with limits of quantification of 0.01 mg L⁻¹.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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