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Statistical evaluation of an analytical GC/MS method for the determination of long chain fatty acids

Pablo Campo [a,*], George A. Sorial [a], Makram T. Suidan [a], Albert D. Venosa [b]

[a] *Department of Civil and Environmental Engineering, University of Cincinnati, 765 Baldwin Hall, Cincinnati, OH 45221-0071, USA*
[b] *The U.S. EPA National Risk Management Research Laboratory Cincinnati, OH 45268, USA*

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Abstract

In-depth evaluation of an analytical method to detect and quantify long chain fatty acids (C_8–C_{16}) at trace level concentrations (25–1000 μg/l) is presented. The method requires derivatization of the acids with methanolic boron trifluoride, separation, and detection by gas chromatography–mass spectrometry. The calibration experiments passed all the tested performance criteria such as linearity, homoscedasticity, and ruggedness. The detection limits and related quantities were computed by applying the method detection limit, and the calibration line approximation. The values obtained by applying the latter approach were more reliable and consistent with the actual statistical theory of detection decisions and yielded the following concentrations: C_8, 87.6 μg/l; C_{10}, 45.2 μg/l; C_{11}, 39.9 μg/l; C_{12}, 37.7 μg/l; C_{14}, 41.4 μg/l and C_{16}, 40.6 μg/l. Two different gas–liquid chromatographic columns were tested and similar results achieved, which shows the ruggedness of the method.

Keywords: Long chain fatty acids; Calibration; Linearity; Detection limit

1. Introduction

Among the different kinds of lipidic biomolecules, the most abundant compounds are fats and oils. Fats are the main constituent of the storage fat cells in animals and plants and, chemically, are triacylglycerols, i.e., carboxylic esters derived from the single alcohol, glycerol. Each fat consists of glycerides derived from diverse carboxylic acids with C_8–C_{18}, being the most abundant long chain fatty acids. The diverse chemical composition of fats initiated their use as raw materials for many and distinct industrial products such as foods, detergents, surfactants, or drying oils [1]. Therefore, there are important economic activities surrounding these compounds, which require transportation, storage, treatment of wastes, etc. For this reason, in a 1997 Federal Register announcement [2], the U.S. Environmental Protection Agency (EPA) issued an opinion that non-petroleum oils (i.e., vegetable oils and animal fats) should not be exempted from regulations that govern the cleanup of oil spills. EPA's Office of Research and Development (ORD) is responsible for conducting research that addresses the issue of non-petroleum oils and to obtain scientifically sound information on the fate and effects of such oils in the environment, with special attention paid to the biodegradability and toxicity of vegetable oil before, during, and after exposure to degrading microbial populations in the aqueous phase.

The principal components of vegetable oils are triglycerides, and their main intermediates during the biological activity are long chain fatty acids [3,4]. Several methods have been proposed for analyzing these organics in seawater and sewage sources [5–9]. These procedures involve either liquid–liquid extraction (LLE) or solid phase extraction (SPE), derivatization of the fatty acids, and gas chromatographic separation and detection. Such investigations involve time-consuming procedures, lack a meticulous statistical study of the chemical measurement process (CMP)

* Corresponding author. Tel.: +1 513 556 3637; fax: +1 513 556 2599.
E-mail address: campomp@email.uc.edu (P. Campo).

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[10], and do not report cumulative blank contributions of palmitic or stearic acid. Hence there is a need for a procedure to analyze fatty acids in water. When dealing with new methods, the first action consists of determining the physical and chemical properties of the target compound, its matrix, and the estimated concentration in which the former is present in the latter. Then the analytical procedure can be designed in order to establish a CMP. In the core of this process, two main issues are essential: precision and accuracy. For this reason, the quantitative potential of the method has to be corroborated by means of an entire evaluation of its efficiency and validation. This step requires the definition of a set of performance criteria. The primary criteria are precision, bias, accuracy, and the detection limit. The secondary criteria are linearity, range, quantification limit, selectivity, sensitivity, and ruggedness ([11], Chapter 13). Not all the performance characteristics are determined in preliminary studies. At this point, the indispensable criteria are precision, which is given by repeatability, linearity, and linear range, estimated from the regression analysis of the calibration curve, and the lowest limits of the method, obtained from blank measurements, low concentration samples or calibration curves [12].

The objective of this work is to carry out a detailed evaluation of a CMP for quantifying long chain fatty acids in water (C_8–C_{16}). Due to the low solubility of these compounds in aqueous matrices [13], a calibration procedure is designed in the parts-per-billion concentration range (μg/l). This protocol consists of derivatization of the fatty acids to obtain the corresponding fatty acid methyl esters (FAMEs), separation by gas chromatography, and detection by mass spectrometry (GC/MS). The derivatization step is required due to the presence of carboxylic groups, which lack of a suitable gas chromatography behavior. The procedure transforms the acids into methyl esters that possess both lower polarity and vapor pressure, improving their separation and quantification by GC [14,15]. The results of the quantification and detection limits will be very useful to predict and optimize an SPE procedure for the analysis of large volume environmental samples [16].

2. Experimental

2.1. Chemicals

The target compounds caprylic (C_8), capric (C_{10}), undecanoic acid (C_{11}), lauric (C_{12}), myristic (C_{14}), and palmitic (C_{16}) acids, and the internal standard, tridecanoic acid (C_{13}), were purchased from Sigma (St. Louis, MO, USA). All the fatty acids were analytical reagent grade with minimum 99% purity. The boron trifluoride (BF_3) methanol solution was acquired from Aldrich (Milwaukee, WI, USA). Methanol, methylene chloride, and hexane were obtained as Optima grade from Fisher Scientific (Pittsburg, PA, USA).

2.2. Standard solutions and derivatization

Two sets of stock solutions were prepared using methanol as solvent: (a) the target compounds (20 and 5 mg/l), and (b) the internal standard (IS, 100 mg/l). Six standard solutions (1000, 400, 200, 100, 50, and 25 μg/l) containing the target compounds were prepared to perform the calibration of the GC/MS. The IS was added at a concentration of 500 μg/l in all of the calibration standards. The solutions were prepared in 2 ml Pyrex® flasks with caps (Corning, NY, USA), by spiking the desired concentration of stock solution in 2 ml of methylene chloride. These samples were evaporated to dryness under a gentle stream of nitrogen. After the evaporation step, 0.5 ml of BF_3 methanolic solution was added and the flask was then heated at 55 °C for 30 min. After cooling, 1 ml of hexane was added and the reaction flask was then shaken for 30 s in a Vortex mixer. The organic extract was transferred to an autosampler vial and analyzed. Derivatization blanks were prepared in the same way but the internal standard was the only spiked compound.

2.3. Chromatographic equipment and experimental conditions

GC/MS analyses were performed with an Agilent (Palo Alto, CA, USA) 6890 Series GC system equipped with a 7683 Series injector and a 5973 Network Mass Selective Detector. One microliter of the extract was injected in pulsed on-column mode in a single taper direct connect liner, 4 mm i.d., obtained from Agilent (Palo Alto, CA, USA). The liner, following an in-house procedure, was deactivated and packed with 5 mg of glass wool before every run. The inlet temperature was 310 °C. The carrier gas was ultra high purity helium.

Two different chromatography columns were tested. The first column was a 30 m HP-5MS (J&W, Palo Alto, CA, USA) (cross-linked 5% Phenyl Methyl Siloxane; 0.250 mm i.d., 0.25 μm film thickness). The GC oven temperature was maintained at 50 °C for the first minute, then ramped to 300 °C at 10 °C/min and kept at 300 °C for 15 min. The flow rate of helium flow was 1 ml/min. The second column was a SP-2380 (Supleco, Bellefonte, PA, USA) (30 m, 0.25 mm i.d., 0.20 μm film thickness). For this column, the GC oven temperature was maintained at 50 °C for the first minute, then first ramped to 170 °C at 5 °C/min and then to 250 at 20 °C/min. It was kept at 250 °C for 15 min. The flow rate of helium flow was 0.9 ml/min.

Detection and data acquisition was performed in selected ion monitoring mode (SIM) under a dynamic mass calibration. Under this mode the mass spectrometer is able to determine a SIM ion value to within 0.1 amu. The target molecular ions for the compounds (C_8–C_{16}) were selected to be (m/z) M^+ $[M-43]^+$, 87, and 74. The mass spectrometer parameters were: interface temperature 300 °C, ion source 230 °C, and quadrupole 150 °C. The ionization energy was 70 eV. The software used for the control of the GC/MS and for data acquisition was Environmental ChemStation G1701CA