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Harmonization of the quantitative determination of volatile fatty acids profile in aqueous matrix samples by direct injection using gas chromatography and high-performance liquid chromatography techniques: Multi-laboratory validation study

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ABSTRACT

The performance parameters of volatile fatty acids (VFAs) measurements were assessed for the first time by a multi-laboratory validation study among 13 laboratories. Two chromatographic techniques (GC and HPLC) and two quantification methods such as external and internal standard (ESTD/ISTD) were combined in three different methodologies GC/ESTD, HPLC/ESTD and GC/ISTD. Linearity evaluation of the calibration functions in a wide concentration range (10–1000 mg/L) was carried out using different statistical parameters for the goodness of fit. Both chromatographic techniques were considered similarly accurate. The use of GC/ISTD, despite showing similar analytical performance to the other methodologies, can be considered useful for the harmonization of VFAs analytical methodology taking into account the normalization of slope values used for the calculation of VFAs concentrations. Acceptance criteria for VFAs performance parameters of the multi-laboratory validation study should be established as follows: (1) instrument precision ($RSD_{INST} \leq 1.5\%$); (2) linearity ($R^2 \geq 0.998$; $RSD_{SENSITIVITY} \leq 4\%$; $RE_{MAX} \leq 8\%$; $RE_{AVER} \leq 3\%$); (3) precision ($RSD \leq 1.5\%$); (4) trueness (recovery of 97–103%); (5) LOD ($\leq$3 mg/L); and (6) LOQ (10 mg/L).

Abbreviations: AD, anaerobic digestion; CRM, certified reference material; FDA, Food and Drug Administration (USA); GC, gas chromatography; ESTD, external standard methodology; FFAP, free fatty acid phase; FID, flame ionization detector; HPLC, high performance liquid chromatography; IS, internal standard compound; ISO, International Standard Organization; ISTD, internal standard methodology; LOD, limit of detection; LOQ, limit of quantitation; OLS, ordinary least-squares; P&T, precision and trueness; r, correlation coefficient; R, recovery rate; R^2, determination coefficient; RE, relative error; RF, response factor; RI, refractive index; RRF, relative response factor; RSD, relative standard deviation; RSD_{INST}, instrument RSD instrument precision; RSE, residual standard error; SS_{calib}, calibration standard solutions; $SS_{Pre\&Tru}$, precision and trueness standard solutions; SS_{stock}, stock standard solution; SST, system suitability testing; USEPA, Unites States Environmental Protection Agency; VFAs, volatile fatty acids; WF/wi, weighting factor; WLS, weighted least-squares.

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1. Introduction

The term volatile fatty acids (VFAs) comprises a group of aliphatic monocarboxylic acids with low-molecular weight and short chain lengths (C2–C7). They have peculiar characteristics such as relatively low volatility, high polarity and a strong hydrophilic character. In fact, they are classified as water-soluble volatile acids because they can be distilled at atmospheric pressure through co-distillation with water despite their high boiling points. The nature and concentration of these organic compounds are of interest because they are natural products from the degradation of organic matter constituting key intermediate metabolites in many biological processes. In this way, particularly, VFAs measurements have a high relevance in the anaerobic digestion (AD) research field. Therefore, monitoring the concentration of VFAs in anaerobic reactors as intermediate compounds in the metabolic pathways of fermentation and methanogenesis is viewed as a key control parameter. To consider the importance of this topic, a Scopus web search in article title/abstract/keywords using the terms "anaerobic digestion" and "volatile fatty acids" reported 2042 results in the period 1990–2015.

A wide range of analytical methods is available for the determination of VFAs in various matrices, wherein GC and HPLC are the most common analytical techniques [1–3]. In fact, the scientific literature contains many papers related to different chromatographic methodologies for the determination of these organic compounds from the original work carried out by James and Martin, who reported firstly the separation of C1–C12 by GC, as early as 1952 [4]. From the analytical viewpoint, it is important to note that samples can be analyzed directly [5,6], and when possible, the direct analysis is always preferable, because of its simplicity. However, also different pre-treatments such as distillation, organic extraction, derivatization and acidification increase the variability in the analytical methodology. Considering the great number of variables affecting the analytical determination of VFAs by chromatographic techniques, the standardization of these methodologies is difficult to achieve.

Method validation is an important requirement in chemical analyses for testing the suitability of methods as well as the capacity of the analyst and laboratory. The results from method validation can be used to judge the quality, reliability and consistency of analytical results. Considering the importance of VFAs measurements and the numerous research groups and laboratories worldwide interested in them, the harmonization of VFAs measurements should be achieved in order to bring together different approaches, experiences and knowledge with analytical methods. In this way, it is important to note that against an in-house validation method, wherever possible and practical, a laboratory should use a method of analysis whose performance characteristics have been evaluated through a collaborative study that should conform an international protocol [7,8]. For these different reasons, the main goal of the present paper is the harmonization of VFAs results, by recognizing, understanding and explaining analytical differences among participants while taking steps to achieve worldwide uniformity in VFAs measurements. Therefore, results from a multi-laboratory validation study are presented including:

- Detailed information on the experimental validation approach.
- Performance characteristics of analytical methodologies reported by the participants.
- Information about the decision of accepting the performance characteristics of the analytical method with respect to its intended use. By this way, minimizing the risk to accept a procedure that is not sufficiently accurate or to reject a procedure that is capable of providing good results.

2. Multi-laboratory validation study

2.1. Validation of VFAs: state of the practice

Validation guidelines, in general, seldomly provide a practical approach to how validation should be carried out in a particular laboratory. There is much information about the criteria of validation to be tested, but it is frequently restricted to theoretical concepts and does not provide any experimental approach. In consequence, it is not always easy for analysts to translate the general concepts into practice considering the type of application, the method requirements and the choice of acceptance criteria. Concerning to VFAs, in spite of the many studies dealing with their measurement, only a few papers include a full study of the performance parameters that characterize the validation of the analytical methodology. On the other hand, neither ISO nor USEPA methods have been published for these organic compounds. Although the Standard Methods Committee approved the GC technique for VFAs measurements (SM 5560D) in 2005 [9], the reported methodology could be considered as inadequate. This is due to include some suggestions that can not be considered as good analytical validation practise: low number of calibration levels ($j = 4$); narrow calibration range (typically, 3.5–350 mg/L); calibration curve using the best fit through zero; acceptance criteria of linearity was based on correlation coefficient (should be higher than 0.995) and a 15–20% of deviation error for each calibration point; and finally, the precision and trueness (P&T) of the methodology were based on single-laboratory data.

A long-standing objective of the AD research community has been to produce comparable results among laboratories through harmonized analytical methods. Although reliable analytical determinations of VFAs are required for the performance evaluation of anaerobic reactors, an interlaboratory study carried out recently involving laboratories working in the AD research field revealed a poor overall performance or "state of the practice" [3]. Among the causes for the poor analytical performance, human errors and inadequate analytical calibration procedures were the major problems observed. In addition, a reference methodology should be necessary to compare the VFAs results obtained by "on-line" anaerobic reactor monitoring using near-infrared spectroscopy (NIRS) technique, but unfortunately the error of prediction was too large for their accurate quantification [10]. These results showed that a good laboratory practice was complicated and a further multi-laboratory study is considered as crucial to improve the analytical reliability of VFAs measurements.

2.2. Organization

Information about this interlaboratory study was sent to laboratories and research groups working in the AD field. There was no attempt to screen participants in any manner, and therefore, all laboratories that expressed their interest to participate were welcomed. The potential candidates with interest in VFAs analysis received a first announcement of this action in October 2013. Of these, 30 laboratories, most of them members of different universities from the EU, agreed to participate in this interlaboratory study before the deadline for the distribution of the materials. The high level of positive responses can be considered as an indication of need for harmonization in the AD research field. The participating laboratories received instruction guidelines and the "validation kit" in February of 2014. Each validation kit contained 18 glass vials containing different aqueous solutions. In addition, each laboratory received the following fungible materials: volumetric flasks, vials for injection and vials to store some solutions to be prepared in the laboratory. The schedule was set to complete the interlaboratory study within 3 months after receiving the samples. Unfortunately,