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Determination of total fatty acids (C_8–C_{22}) in sludges by gas chromatography–mass spectrometry

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Abstract

A simple method for determination of total fatty acids (C_8–C_{22}) in sludges down to detection limits as low as 3–5 μg/g is reported. They are extracted with dichloromethane and esterified using BF_3 methanolic solution followed by an 11 min run using gas chromatography–mass spectrometry with selected ion monitoring (GC–MS-SIM). Tridecanoic acid was used as a surrogate standard. The applicable concentration range was 8 to 3000 μg/g. The method was validated by standard addition methodology. The method was applied satisfactorily to the determination of these compounds in sludges from a waste water treatment plant in Granada (Spain).

Keywords: Sludge analysis; Fatty acids

1. Introduction

Fatty acids have long been associated to cleaning tasks and because of the nature of such tasks and of the involved materials (coming from vegetables or animals) the idea that their environmental impact should not be unreasonable seems to be deeply rooted.

Some doubts however can arise however on subjects such as the potential interactions between Ca–Mg fatty acid salts and aerobic or anaerobic microorganisms. For instance the Ca+Mg–Na ratio is substantially higher in anaerobically digested sludges than in either treated or untreated waters. Organic matter, including soaps, could be precipitated as Ca–Mg salts in waste water treatment plants (WWTPs) becoming integrated in the solid/sludge fractions coming out of the WWTPs escaping thus the biological treatments and/or chemical processes. Apparently, no harmful accumulations or aquatic toxicities seem to have been reported although some caution on these issues is warranted.

Methods to analyze fatty acids in different matrices such as environmental samples, biological samples, industrial samples, food samples, etc., have been proposed. They have in common an extraction step, a derivatization step and as a general rule they require a lot of time, with the exception perhaps of the one proposed by Muskiet et al. for biological samples involving a direct sample derivatization/esterification [1].

The analysis of fatty acids from all kinds of sludge samples, has usually been carried out through ''Soxhlet'', a solid–liquid extraction method [2–8]. The extraction method is time-consuming with rather

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high consumption of organic solvent. Other authors report on liquid–liquid extraction [9] but this is a tedious method implying 83 steps. Solid–liquid extraction followed by solid-phase extraction [10] requires only two extraction steps. Other techniques, such as supercritical carbon dioxide extraction, have also been used [11].

A new method for the analysis of total fatty acids (C_8–C_{22}) in sludges is presented here, whereby the dry sludge acidified suspension requires only 6 h of mechanical stirring in a sonicated bath, followed by a liquid–liquid extraction, an esterification step [12] and monitoring of the fatty acid methyl esters (FAMEs) by gas chromatography–mass spectrometry (GC–MS). This work features our findings on this subject in the Granada City WWTP.

2. Experimental

2.1. Apparatus and software

All chromatographic measurements were performed with a Hewlett-Packard system made up by a 5890 gas chromatograph fitted with a HP 7673 autosampler, a splitless injector for capillary columns and a 5971 mass spectrometer with electron impact (EI) mode of 70 eV as ionization source and quadrupole mass filter and a HP DA-5100 data system.

A Statgraphics 6.0 software package [13] was used for regression analysis (linear model). An Alamin software package [14] was used for the statistical analysis of data.

2.2. Reagents

The fatty acids (caprylic acid C_8, capric acid C_{10}, lauric acid C_{12}, myristic acid C_{14}, palmitic acid C_{16}, oleic acid $C_{18:1}$, stearic acid C_{18}, arachidic acid C_{20} and behenic acid C_{22}) were analytes; tridecanoic acid C_{13}, was the surrogate standard and 14% boron trifluoride solution in methanol was used for esterification. All of them came from Sigma (St. Louis, MO, USA) and were of analytical-reagent grade 99%.

The stock solutions of fatty acid containing 100 mg l^{-1} were prepared in 500-ml volumetric flasks, by dissolving 50.0 mg of the compound in methanol 96% (v/v) (Panreac, Barcelona, Spain).

A standard solution of 100 mg l^{-1} of tridecanoic acid was used as a surrogate standard.

All solutions were stored in dark bottles at 4°C, remaining stable for at least six months.

2.3. Sample treatment

Sludge samples were dried at 60°C in an electric oven. They were pulverized and pestled in an agatha mortar, and finally homogenized in a analytical mill.

2.4. Extraction method

Thirty mg of dry sludge was suspended in 500 ml of deionized water and HCl (1:1) was added to bring the pH to 1. The acid suspension was mechanically stirred in a sonicated bath for no more than 6 h. Then, the samples were transferred to a separatory funnel and 50 ml of methylene chloride were added. The mixtures were shaken for 1 min and then the organic phase was collected. The extraction was repeated once again with 50 ml of organic solvent. The extracts were mixed, dehydrated with anhydrous sodium sulfate, filtered and concentrated to dryness in a rotary vacuum evaporator. The extract was dissolved in 3.0 ml of methanol and 1 ml of methanolic solution of 1 mg l^{-1} tridecanoic acid, used as surrogate standard. The fatty acids mixture solution was collected in a clean dry test tube being then ready for the esterification step.

2.5. Esterification method

2.0 ml of 14% BF_3 methanolic solution was added to the fatty acids mixture methanolic solutions and placed in a 70°C water bath for 3 min. Then, 1.0 ml of deionized water was added followed by cooling to stop the reaction. The FAMEs were extracted from the aqueous methanol phase by adding 1 ml of methylene chloride and shaking the test tube for 1 min to favor mixing. Two layers formed, the methylene chloride layer was drawn off with a Pasteur pipette and transferred to another test tube while the aqueous methanol phase was extracted twice again with 1.0 ml of methylene chloride. The extracts were then mixed, dehydrated with anhydrous sodium

sulfate, filtered and placed in a 10-ml volumetric flask, completing using methylene chloride.

2.6. GC–MS conditions

The column was a HP1 fused-silica capillary (30 m×0.25 mm I.D., 0.25 mm film thickness) coated with methyl silicone gum phase. Carrier gas was helium (purity 99,999%). A 2-μl aliquot of the extract containing the methyl esters was injected by the autosampler in the injector using splitless mode with the split closed for 2 min. Injector temperature, 200°C; detector temperature, 280°C; oven temperature programmed from 75°C (1 min) to 270°C at 30°C min^{-1}, the final temperature held for 7 min.

The selected ions of the compound for SIM mode operation were m/z 55, 74 and 87. The concentrations of the fatty acids were calculated by the internal standard method.

2.7. Calibration

Five methanolic solutions containing mixtures of (C_8–C_{22}) fatty acids of known concentrations (0, 2.5, 5.0, 7.5, 10.0 mg l^{-1}) and 1 mg l^{-1} of the selected surrogate standard C_{13}, were esterified as described in Section 2.5 and directly injected into the gas chromatograph. A calibration graph was constructed for each fatty acid.

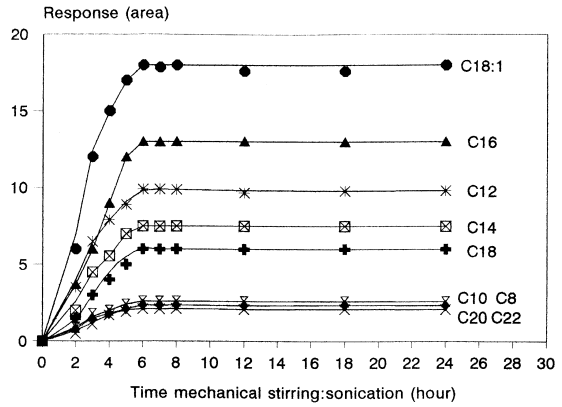

Fig. 1. Mechanical stirring:sonication time versus area for each fatty acid.

3. Results and discussion

We decided to study the fatty acid contents in sludge samples, because as seen from the bibliography selected by us in Table 1, extraction methods are tedious and lengthy and analyses are time-consuming.

Dry sludge amounts of 10, 20, 30, 40, 60 mg suspended in 500 ml of deionized water were tested. The samples above 30 mg of dry sludge presented a significant interface when mechanical stirring and sonicated in methylene chloride. Therefore, 30 mg of sludge in 500 ml of deionized water seem to be a reasonable approach.

Mechanical stirring and sonication lasted for

Table 1
Bibliography extract of analysis of fatty acids in sludges

Sample	Extraction method	Time	Technique
Domestic sewage sludge [2]	Soxhlet	70 h	GC–MS
Alkaline lagoon sediment [3]	Soxhlet	36 h	GC–FID, MS
Domestic sewage sludge [4]	Soxhlet	70 h	HPLC
Estuary sediment [5]	Soxhlet	NR	HPLC, GC
Raw sewage sludge [6]	Soxhlet	24 h	GC–FID
Sediment near pulp mill [7]	Soxhlet	10 h	GC–ECD
Sewage slugde [8]	Soxhlet	17 h	GC–MS
Sediment [9]	Cent, LLE, SPE	NR	GC–MS
Soil lipids [10]	SPE	NR	GC–MS
Sediment near pulp mill [11]	SFE	15 min	GC–ECD
Proposed method	US, LLE	6 h	GC–MS

NR, Not reported; Cent, centrifugation; LLE, liquid–liquid extraction; SPE, solid-phase extraction; SFE, supercritical fluid extraction; US, ultrasound.

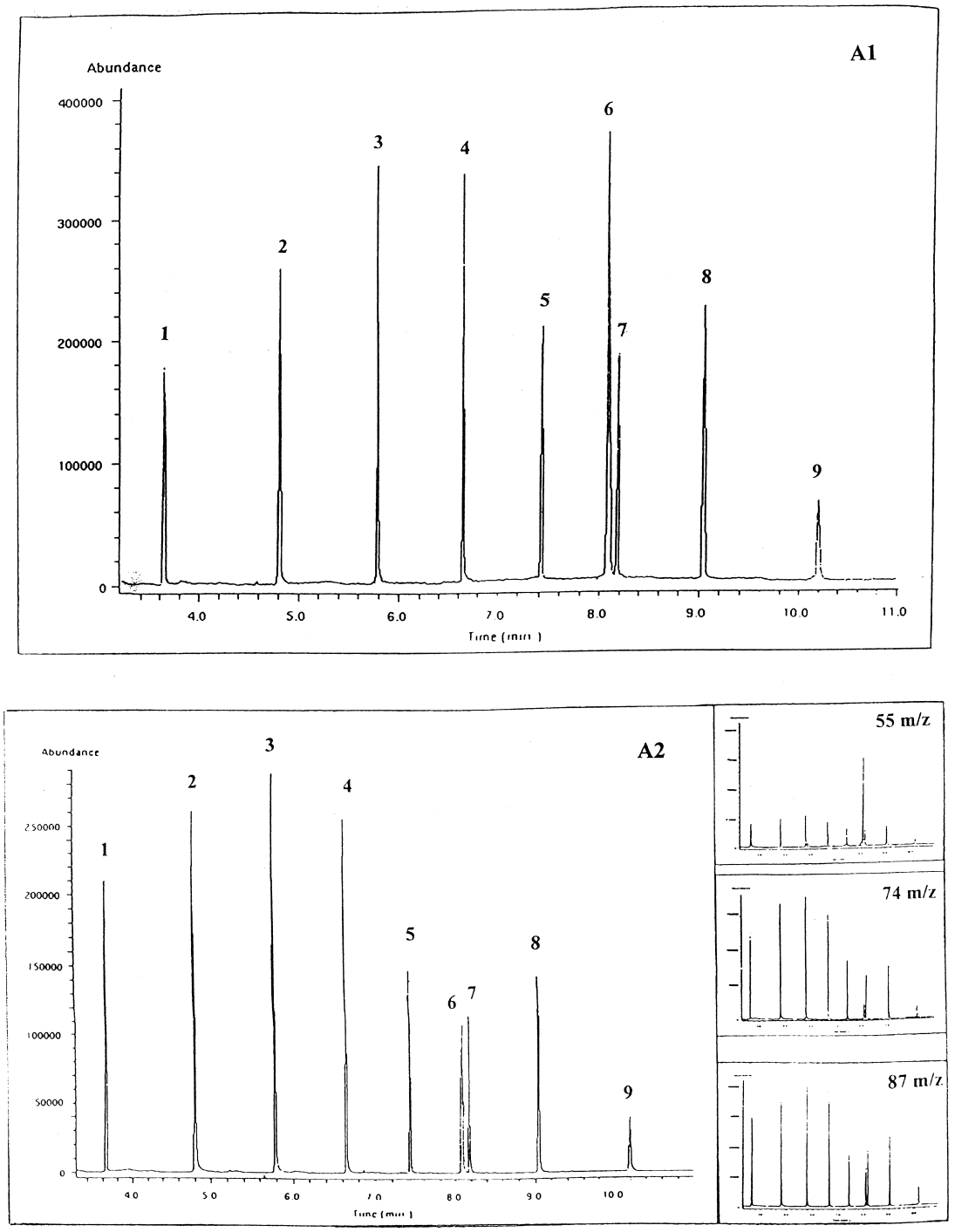

Fig. 2. Typical chromatogram of FAMEs. (A) Standard sample of FAMEs (40 mg l^{-1}); (B) real sample of dry sludge from the WWTP in Granada City (Spain) with concentration as stated in Table 4. FAMEs: 1, C_8; 2, C_{10}; 3, C_{12}; 4, C_{14}; 5, C_{16}; 6, $C_{18:1}$; 7, C_{18}; 8, C_{20}; 9, C_{22}. (A1) and (B1) are total ion current chromatograms. (A2) and (B2) are a single ion monitoring chromatogram at m/z 55, 74 and 87 and individual chromatograms at m/z 55, 74 and 87.

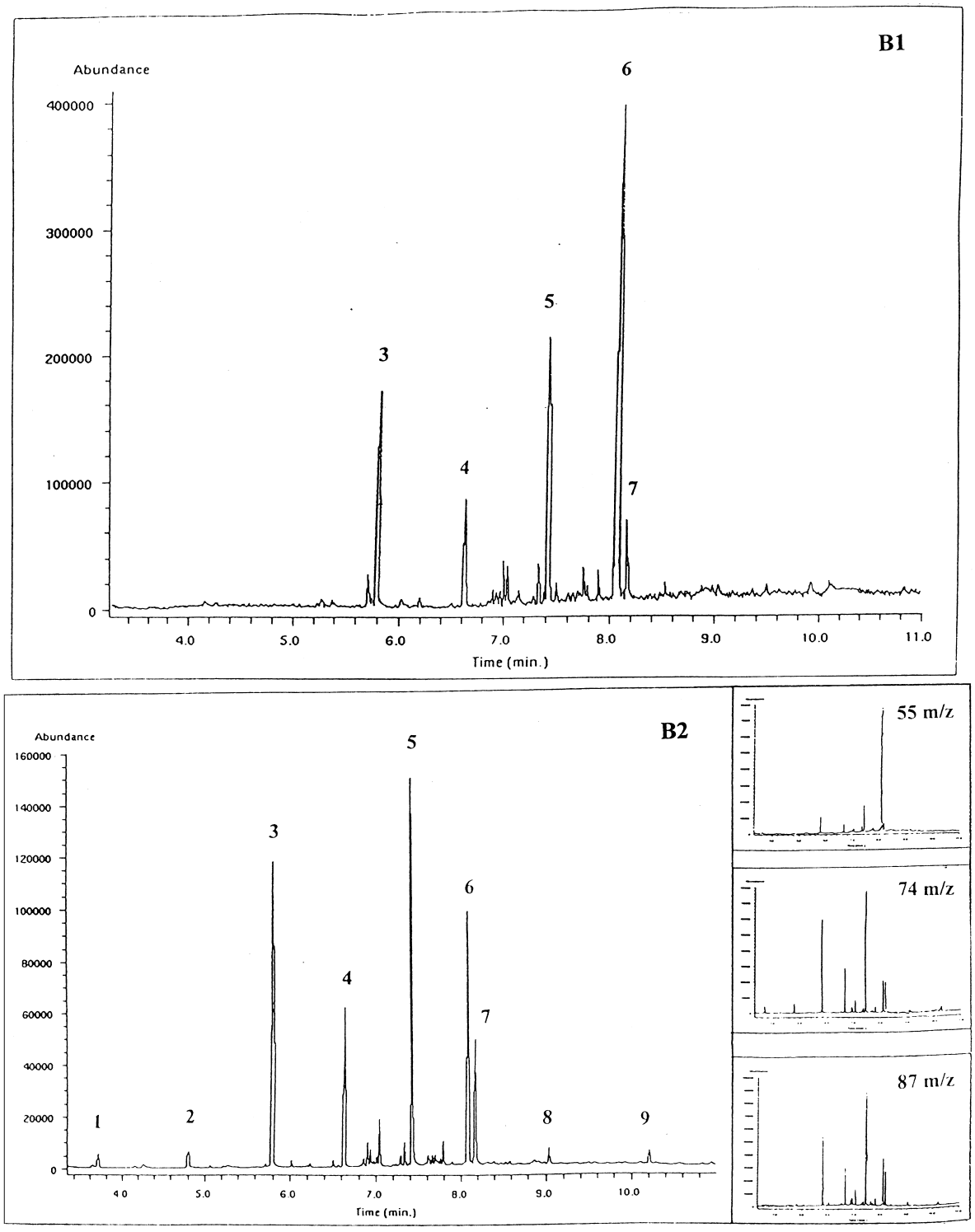

Fig. 2. (*continued*)

periods between 3–24 h, with no more than 6 h required to achieve the maximum area in the chromatograph because with longer time there was no significant improvement in the signals from the samples (Fig. 1).

The extraction method for the acid suspension, reported here was a liquid–liquid extraction. Methylene chloride was the most adequate of the tested organic solvents. A water–methylene chloride ratio of (10:1) was found to be appropriate enough for preconcentrating the analytes to the required final concentration thus minimizing the use of significant amounts of organic solvent.

Behavior of fatty acids over a pH range was investigated. As fatty acids are weak acids with high pK_a values, the chromatographic signal remains constant until dissociation of the chemical takes place at higher pH values and the signal decreases. Decreased signals represent a diminution in the extraction efficiency because the dissociate form remains in the water phase. Acidification to pH 1 of the solutions is carried out by adding a few drops of hydrochloric acid.

Typical chromatograms obtained from the standard sample and the real sample under the above described set-up conditions are shown in Fig. 2A Fig. 2B, respectively. Only 11 min were necessary to complete an analysis.

In a SIM analysis a high mass number and a high intensity were chosen in order to obtain good sensibility and to prevent interferences.

The mass spectrums of the FAMEs were carried out in scan mode.

The molecular ions appear at the corresponding molecular mass. The base peak corresponds to the McLafferty rearrangement and appears at m/z 74, except in the case of oleic methyl ester (base peak, m/z 55). A relevant peak corresponding to a specific eight-center rearrangement and H shift appears at m/z 87 in all cases. Other specific peaks showed a lower abundance. Due to its higher abundance we selected m/z 74 as a target ion and m/z 55 and 87 as qualifier ions for the SIM mode analysis.

3.1. Analytical parameters

Calibration graphs for samples treated according to the analytical procedure above described were made using SIM mode. They are linear for the concentration range 8–3000 μg g^{-1} of each fatty acid. In order to check the linearity of the calibration standard the lack-of-fit test Analytical Methods Committee [15] was applied to two replicates and three injections of each standard. The results for the intercept (*a*), slope (*b*), correlation coefficient (*r*), linear dynamic range (LDR) and probability level of lack-of-fit test [P_{lof} (%)] are summarized in Table 2. Thus, the data yield a good linearity within the stated range.

There is no agreement about how to get the detection limits (DLs) and quantification limits (QLs)

Table 2
Analytical parameters

Fatty acid	*a*	*b* (l mg^{-1})	*r*	LDR (μg g^{-1})	LOF	DL (μg g^{-1})	QL (μg g^{-1})
C_8	0.000	0.899	0.999	11–3000	0.62	3	11
C_{10}	−0.002	0.964	0.999	14–3000	0.31	4	14
C_{12}	−0.001	0.955	0.999	11–3000	0.87	3	11
C_{14}	−0.001	0.926	0.999	14–3000	0.63	5	14
C_{16}	0.001	0.948	0.999	8–3000	0.29	3	8
C_{18}	0.001	0.957	0.999	13–3000	0.58	4	13
$C_{18:1}$	0.002	0.896	0.999	8–3000	0.15	5	8
C_{20}	−0.001	0.829	0.999	13–3000	0.70	4	13
C_{22}	0.001	0.759	0.999	17–3000	0.16	4	17

a, Intercept; *b*, slope; *r*, correlation coefficient; LDR, linear dynamic range; LOF, lack-of-fit test (*p* value); DL, detection limit=$3s_{c0}$; QL, quantification limit=$10s_{c0}$ (s_{c0}; standard deviation from ''zero concentration'' obtained from linear regression. These parameters were obtained from 0.0, 2.5, 5.0, 7.5 and 10.0 mg l^{-1} solutions of each fatty acid. Two experimental replicates and three instrumental replicates (injections) of each fatty acid.

from the blank standard deviation in GC, in contrast with other analytical techniques. Frequently, IUPAC recommendations are not strictly used.

We believe that the method that we have applied [16] for calculating DLs and QLs in pesticides in water is more in line with the IUPAC recommendations. It relies on studying the blank standard deviation in an interval of time corresponding to the peak width in its base, extrapolated to zero concentration.

3.2. Validation and applications of the method

The proposed method was applied to a sludges from a Municipal WWTP of Granada city (Spain). We found fatty acids in these sludges. The validation of the proposed method to sludge samples was carried out by using standard addition methodology [17]. Three experiments were required to obtain the data set necessary to apply the proposed statistical

Table 3
Statistical parameters from standard addition validation

Fatty acid		Regression parameters			Slope comparisons		Analyte content		
		a	b	S_{yx}	b_p	t_{cal}	C_{SC}	C_{AC}	t_{cal}
C_8	SC	0.00	0.89	0.0036			0.285		
	AC	0.77	0.91	0.0096	0.90	1.82		0.286	1.10
	YC	0.00	0.09	0.0003					
C_{10}	SC	0.00	0.96	0.0076			0.370		
	AC	1.04	0.92	0.0066	0.94	0.79		0.366	0.27
	YC	0.00	0.10	0.0002					
C_{12}	SC	0.00	0.96	0.0098			5.75		
	AC	16.7	0.97	0.1245	0.96	0.18		5.74	0.28
	YC	0.00	0.10	0.0002					
C_{14}	SC	0.00	0.93	0.0076			2.97		
	AC	8.25	0.93	0.0096	0.93	0.56		2.97	0.06
	YC	0.00	0.10	0.0002					
C_{16}	SC	0.00	0.95	0.0076			7.09		
	AC	20.2	0.96	0.0178	0.95	0.77		7.10	0.42
	YC	0.00	0.10	0.0005					
C_{18}	SC	0.00	0.96	0.0080			2.25		
	AC	6.46	0.96	0.0093	0.96	0.42		2.25	0.34
	YC	0.00	0.10	0.0001					
$C_{18:1}$	SC	0.00	0.90	0.0076			12.6		
	AC	33.8	0.88	0.0150	0.90	1.98		12.6	0.45
	YC	0.00	0.09	0.0006					
C_{20}	SC	0.00	0.82	0.0076			0.391		
	AC	0.96	0.81	0.0089	0.82	0.70		0.391	0.08
	YC	0.00	0.08	0.0005					
C_{22}	SC	0.00	0.76	0.0076			0.389		
	AC	0.88	0.75	0.0071	0.75	1.73		0.386	0.20
	YC	0.00	0.08	0.0005					

SC, Standard calibration; AC, addition calibration; YC, Youden calibration; a, intercept; b, slope; S_{yx}, regression standard deviation; b_p, pooled slope of AC and SC; C_{SC}, analyte concentration calculated by standard calibration; C_{AC}, analyte concentration calculated by addition calibration; t_{cal}, statistical for t-test.

Table 4
Concentration of fatty acids presents in Granada city dry sludge

Fatty acid	Concentration (mg g^{-1})
C_8	0.288±0.003
C_{10}	0.353±0.003
C_{12}	5.76±0.06
C_{14}	2.97±0.04
C_{16}	7.09±0.09
C_{18}	2.25±0.03
$C_{18:1}$	12.58±0.13
C_{20}	0.384±0.005
C_{22}	0.384±0.004

protocol. For each fatty acid the same analytical procedure was applied: (a) standard calibration (SC) as described above; (b) standard addition calibration (AC) obtained by spiking five samples with a mixture of standard fatty acids with final concentrations for each fatty acid in this sample: 0.000, 0.025, 0.050, 0.075, 0.100 mg g^{-1}; (c) Youden calibration (YC): a calibration curve was made with the Youden method, increasing amounts of sample mass are checked (0.25, 0.50, 0.75, 1.00 mg). The parameters obtained from these three procedures are shown in Table 3. The p value for the Student t-test of the representative values of slope deduced for the SC and AC methods show in all cases (p value$>$ 0.05) the similarity of these slopes. The p value for the Student t-test to compare the concentrations of analyte deduced for the SC and AC methods shows in all cases (p value$>$0.05) the similarity of these concentrations and the recoveries in all cases were close to 100%. We concluded than our method is accurate. On the other hand, the non-existence of an intercept in the YC implies the absence of a matrix effect validating the method.

The method was applied to the determination of fatty acids in dry sludge from a WWTP in Granada City (Spain). Ten representative portions of a batch sample of dry sludge are analysed and the results are shown in Table 4.

4. Conclusions

A simple method for the analysis of total fatty acids in sludges by GC–MS is presented. The extraction time is drastically reduced using ultrasound. Since extraction time are shorter it may be that this technique could be interesting enough to people involved with wastewater disposal monitoring. The method was applied satisfactorily to sludge samples from a WWTP in Granada City (Spain).

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