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1 **Development of a simple, low-cost and rapid thin-layer chromatography method for**  
2 **the determination of individual volatile fatty acids**

3  
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7  
8 **Abstract**

9 In this paper, a new thin-layer chromatography (TLC) method for the determination of  
10 individual volatile fatty acids (VFA) is presented. The experimental procedure is based on the  
11 derivatization of VFA with activating agents and a naphthalene-based amine to form an  
12 amide derivative, followed by fast TLC separation of these VFA derivatives and visualization  
13 with phosphomolybdic acid at room temperature. Quantification is then performed by  
14 densitometric measurements using a free software after TLC pictures in a home-made black  
15 box for homogeneous and reproducible lighting of the plates. Optimization of the  
16 experimental conditions enables fast determination (20 minutes for 3 samples) of VFA with  
17 detection limits (1.5-2.5 mg.L<sup>-1</sup>) and relative standard deviations (4.6-6.7 %) satisfactory  
18 enough for our analytical purpose using low-cost instruments with easy to find materials and  
19 software. The protocol was then applied to real samples (aqueous extracts of sewage sludge  
20 composts) and results were compared with gas chromatography analysis with good  
21 agreement between the two methods.

22 **Keywords:** Volatile fatty acids, thin-layer chromatography, low-cost, environmental analysis.  
23  
24

25 **1. Introduction**

26 Anaerobic digestion of biomass leading to biogas production has raised considerable  
27 attention over the past years, with applications such as wastewater [1], sewage sludge [2],  
28 organic wastes [3] or landfill leachates [4]. The challenge for anaerobic digestion processes  
29 is to maintain a stable fermentation process and consistent biogas production, and the  
30 monitoring of several parameters such as pH, alkalinity or biogas production rate has been  
31 proposed to this end [5]. However, it has been widely acknowledged that volatile fatty acids  
32 (VFA) concentrations (in particular acetic, propionic, isobutyric, butyric, isovaleric and valeric  
33 acid) provide the most meaningful measure for assessing the effectiveness of the digestion  
34 process [6]. Indeed, VFA are produced and consumed during the main stages of anaerobic

35 digestion processes (hydrolysis, acidogenesis, acetogenesis, and methanation), and this  
36 parameter therefore reflects best the metabolic state of the biochemical process.

37 VFA measurement can provide two types of information: total VFA (sum of all VFA  
38 expressed as mg/L as acetic acid) or speciation of individual VFA. Total VFA is often used as  
39 control parameter as relatively simple analytical methods such as titration [7], near-infrared  
40 spectroscopy [8] or spectrophotometry [9] are available for research or operational  
41 laboratories. Despite this simplicity, total VFA measurements can be insufficient for precise  
42 optimization of anaerobic digesters, as understanding of the composition of VFA during all  
43 stages of the digestion process is also a key point. Indeed, VFA speciation results enable  
44 operators to monitor the current state of the anaerobic digesters and adjust the operational  
45 strategies to improve performance. Accumulation of propionate is a common indicator of  
46 stress in an anaerobic digester [10-12], and traditional non-differentiating methods like  
47 titration cannot give early alert for this potential stress that may lead to detrimental bacterial  
48 community change and process failure. Moreover, analytical techniques which give individual  
49 VFA composition also show strong interests for bioplastic production field which rely on VFA  
50 as carbon source, for example for polyhydroxyalkanoates production [13]. It can thus be  
51 concluded that there is an urgent need for simple analytical tools for speciation of VFA in  
52 operational laboratories monitoring anaerobic digesters performances.

53 Traditionally, speciation of VFA is performed by gas chromatography, liquid chromatography  
54 or capillary electrophoresis [14-17]. These methods based on separation techniques can all  
55 provide reliable results, but their relative complexity requiring skilled personnel and high  
56 purchase and operational costs (separation columns, eluents, gases...) strongly limit their  
57 use for operational laboratories which prefer simple and low-cost analytical tools for routine  
58 analysis. Among separation techniques, Thin-Layer Chromatography (TLC) is the oldest one  
59 and certainly the simplest and least expensive tool in analytical chemistry. Many studies  
60 have already proposed TLC as an alternative to more modern and complex chromatographic  
61 system for quality control and fast quantitative or semi-quantitative determination [18-21].  
62 Due to its simplicity, sample clean-up can be minimized, and cross-contaminations or  
63 columns clogging are avoided, which are significant advantages for complex samples such  
64 as sewage sludge or wastewater.

65 Our research group has developed an original procedure for the determination of total VFA  
66 by batch procedure or microplate assays [22,23]. The analytical methodology is based on a  
67 specific derivatization of VFA via a two-step mechanism: (i) activation step with two activating  
68 agents at acidic pH; (ii) amidation step with a fluorescent amine at basic pH. This  
69 methodology was also applied for the development of a multi-syringe chromatography  
70 system for the determination of individual VFA [24]. This system was based on the  
71 separation of VFA derivatives on a C<sub>18</sub> monolithic column and quantification by UV or

72 fluorescence detection, and it could serve as a relatively simple and low-cost on-line analyzer  
73 compared to complex on-line chromatographic systems.

74 In the present paper, a very simple and rapid TLC method for the determination of individual  
75 volatile fatty acids is presented. This method is intended as an analytical tool for operational  
76 laboratories monitoring anaerobic digesters performances or other environmental samples  
77 who cannot afford HPLC or GC but require routine, rapid and low-cost measurement of  
78 individual VFA (without sending samples to external laboratories). The procedure is based on  
79 previously-mentioned derivatization of VFA combined with fast TLC separation of VFA  
80 derivatives and visualization with phosphomolybdic acid at room temperature. Development  
81 and application of this procedure for quantitative determination of VFA are presented herein.

82

## 83 **2. Experimental**

84

### 85 **2.1 Reagents, solutions and materials**

86 All chemicals and solvents were of analytical or HPLC reagent grade and used without  
87 further purification. Cyclohexane and ethyl acetate were purchased from Sigma-Aldrich.  
88 “EDC solution” was a solution of the activating reagent N’-(3-dimethylaminopropyl)-N-  
89 ethylcarbodiimide (EDC) (Sigma-Aldrich) prepared at 100 g.L<sup>-1</sup> in absolute ethanol (EtOH)  
90 (Sigma-Aldrich). “HOAT+EDAN solution” was a solution of the secondary activating reagent  
91 7-aza-1-hydroxybenzotriazole (HOAT) (Genscript Corporation, Piscataway, USA) at 6 g.L<sup>-1</sup>  
92 and the derivatizing reagent N-(1-naphthyl)ethylenediamine (EDAN) (Sigma-Aldrich) at 2 g.L<sup>-1</sup>  
93 <sup>1</sup>, prepared as a mixed solution in ultrapure water (Millipore, resistivity >18 MΩ cm) with final  
94 pH set at 3.6. “KH<sub>2</sub>PO<sub>4</sub> solution” was made of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>)  
95 prepared at 250 mM in NaOH 0.35 M. “PMA solution” was prepared by dissolving  
96 phosphomolybdic acid hydrate (PMA) (Sigma-Aldrich) at 3 % w/v in water. Stock solutions of  
97 carboxylic acid standards (5000 mg L<sup>-1</sup> for acetic acid, 1500 mg L<sup>-1</sup> for other VFA) (Acros  
98 Organics, Geel, Belgium) were prepared in ultrapure water.

99 Thin layer chromatography aluminum plates (20x20 cm, 0.25 mm layer thickness) precoated  
100 with silica gel 60F-254 were obtained from Merck (Darmstadt, Germany) and cut at adequate  
101 dimensions for intended use (6x6 cm). Spots were applied on the plates at 1.3 cm height  
102 from lower edge of the plate and were separated by a minimum distance of 0.8 cm (6 spots  
103 possible on a single plate).

104

### 105 **2.2 Samples**

106 Real samples used for validation were aqueous extracts of composts (extraction protocol  
107 described in Supplementary material) made by mixing anaerobically digested sewage sludge  
108 (from Marseille’s sewage sludge treatment plant) with green wastes (1/3-2/3, v/v). Ages of

109 the composts were between 1 month (AN1) and 6 months (AN 5). All samples were filtered  
110 through 0.45  $\mu\text{m}$  membrane filters (PES membrane, Millipore, France).

111

### 112 **2.3 Analytical protocol for VFA determination**

113 In a 2 mL glass vial were added 300  $\mu\text{L}$  of "HOAT+EDAN solution", 250  $\mu\text{L}$  of sample and 40  
114  $\mu\text{L}$  of "EDC solution". The vial was stirred and left to react for 5 minutes. 75  $\mu\text{L}$  of " $\text{KH}_2\text{PO}_4$   
115 solution" were then added and the vial was again left to react for 1 minute after proper  
116 stirring. 3  $\mu\text{L}$  of the reaction mixture was then manually spotted on the TLC plate using 3  $\mu\text{L}$   
117 micropipettes (Brand) and the spots were dried with a simple hair-dryer set at minimum  
118 heating power until evaporation of water. The plates were developed vertically for 5 min  
119 (solvent front at about 0.5 cm from upper edge of the plate) at room temperature (22°C) in a  
120 100 mL polypropylene disposable tank equipped with a plastic top (Tecan) in order to  
121 minimize organic solvent volumes used for development (1 mL is enough in these tanks;  
122 classical TLC development chambers with higher volumes can be used). Mobile phase was a  
123 mixture of ethyl acetate and cyclohexane (60:40, v/v). After development, the plates were  
124 dried with the hair-dryer for 1 min. Spots were visualized by dipping the plate in the PMA  
125 solution for 10 s (in a 100 mL polypropylene disposable trough) and drying the plate on  
126 absorbent paper. A picture of the plate was taken immediately with a smartphone in a home-  
127 made black box protected from ambient light with a hole for smartphone camera.  
128 Homogeneous and reproducible lighting of the plates in the box was provided by a LED strip  
129 (12 V DC, 400 Lm, 24 LED/m) cut at the dimensions of the box and stuck on the upper part  
130 of the box. Drawing of the box on CAD software is presented in supplementary material  
131 section. Quantitative determination of VFA was performed on a personal computer using the  
132 open-source ImageJ software (National Institutes of Health) and its densitometric analysis  
133 tool. Pictures were analyzed without modification using a constant rectangular selection size  
134 between calibration plates and sample plates. Gel analysis tools were used to trace  
135 densitometric histograms of each VFA lane and peaks areas were calculated using the wand  
136 tool (results can be saved as an excel file for further analysis).

137

### 138 **2.4 Retention factors and resolution between VFA spots**

139 The retention factor  $R_f$  was determined directly on the TLC picture and was defined as the  
140 ratio of the distance traveled by the center of a spot to the distance traveled by the solvent  
141 front.

142 The resolutions between two neighboring spots A and B ( $R_{S(A/B)}$ ) were calculated on the  
143 densitometric histograms given by the gel analysis tools of Image J. Resolutions were  
144 calculated as the ratio between the difference of distance of peaks maximum and the  
145 average of peak widths at base.

146

## 147 **2.5 Gas chromatography analyses**

148 VFA were determined under classical GC conditions using a Varian CP-3800 gas  
149 chromatograph (GC) with a free fatty acid phase (FFAP) 25 m fused silica capillary column of  
150 0.1  $\mu\text{m}$  film thickness and an i.d. of 0.25 mm (Varian CP-WAX 58-CB). The GC oven  
151 temperature was programmed to be incremented from 115 to 140  $^{\circ}\text{C}$  within 5 min, and a final  
152 temperature of 140  $^{\circ}\text{C}$  was then held for 1.25 min. The injector and detector temperature  
153 were set at 290  $^{\circ}\text{C}$  and 300  $^{\circ}\text{C}$ , respectively. Helium was used as carrier gas at a pressure of  
154 1.4 bar. A flame ionisation detector (FID) was used and quantification was performed using  
155 internal standard method. Limits of detection between 3  $\text{mg L}^{-1}$  (valeric acid) and 10  $\text{mg L}^{-1}$   
156 (acetic acid) were obtained. All sample analyses were performed in duplicate

157

## 158 **3. Results**

159

160 Experimental conditions for the derivatization of VFA were similar as those used in a  
161 previous study with the Multi-Syringe Chromatography set-up [24]. The only differences are  
162 the concentrations of the reagents (100  $\text{g.L}^{-1}$  for EDC and 2  $\text{g.L}^{-1}$  for EDAN) which have  
163 been increased in order to gain sensitivity. The main experimental optimizations for the work  
164 described here are related to the TLC application which gives full interest to the analytical  
165 protocol and its intended use.

166

### 167 **3.1 Visualization solution of the spots**

168 Many visualization methods (or stains) can be used for TLC, which can be compound  
169 specific (Dragendorff reagent, ninhydrin...) or universal (iodine, vanillin, phosphomolybdic  
170 acid...), with varying preparation complexity and hazardous nature of reagents (strong acids  
171 or toxic reagents). As the method is intended for operational laboratories, visualization  
172 method with adequate sensitivity, simple preparation procedure and relatively harmless  
173 reagents is preferable. Phosphomolybdic acid (PMA) was thus selected based on these  
174 criteria, as it is a well-known, sensitive and cheap reagent with low toxicity and which doesn't  
175 require strong acids for the preparation of the visualization solution. Usually, PMA is used as  
176 a solution in EtOH and analytes are visualized as dark green to black spots after heating with  
177 a heat gun or a hot plate. In our first experiments, we noticed that PMA solution in water was  
178 also suitable for visualization of our VFA derivatives spots and that no heating was required  
179 after dipping plates in aqueous PMA, which prevents risks associated with the use of heating  
180 gun and flammable organic solvents. Moreover, naked-eye comparison of spots visualized  
181 with PMA in EtOH or PMA in water (Figure 1) proved that aqueous PMA was the best choice  
182 for our application as the spots were much more visible and darker.

183

### 184 **3.2 Mobile phase selection and retention factors**

185 Many organic solvents are used for TLC development with silica gel plates (the most  
186 common and least expensive stationary phase), including highly toxic solvents such as  
187 chloroform, benzene, toluene or hexane. Nowadays, a classical mixture of organic solvents  
188 used for TLC development is cyclohexane/ethyl acetate, due its good efficiency to separate  
189 compounds with various polarities and functional groups, and also to its moderate toxicity  
190 compared to previously-mentioned solvents. Mobile phases with a mixture of these two  
191 solvents in various proportions were optimized on a VFA solution containing acetic,  
192 propionic, butyric and valeric acids at 100 mg.L<sup>-1</sup> (Figure 2). Resolutions between each VFA  
193 were calculated accurately on densitometric histograms plotted from the TLC pictures and  
194 are given in Table 1. As can be seen, mobile phase made with ethyl acetate/cyclohexane  
195 with volume composition of 60:40 provided the best results with optimal separation between  
196 the closest spots (propionic, butyric and valeric acid). Addition of other classical TLC mobile  
197 phase components like formic acid, diethylamine, ethanol or ammonium hydroxide didn't  
198 improve results. Retention factors with this mobile phase (60:40, v/v) were also calculated for  
199 comparison with real samples: acetic acid: 0.24; propionic acid: 0.43; butyric (+isobutyric)  
200 acid: 0.55; valeric (+isovaleric) acid: 0.64.

201

### 202 **3.3 Interferences**

203 The optimized protocol with TLC separation was applied to a wide range of organic  
204 compounds likely to be detected under the reaction conditions such as other carboxylic acids  
205 (formic, lactic, tartaric, succinic, oxalic, malic, maleic, citric, pyruvic, benzoic acid),  
206 carbohydrate (glucose) or amino acids (glycine, alanine, cysteine). Spots of potential  
207 derivatives or native compounds visualized at the end of the protocol were compared to VFA  
208 spots (comparison of R<sub>f</sub> and intensity). Most of potential interfering compounds didn't lead to  
209 any spots after derivatization and TLC visualization. Only formic, lactic and succinic acid  
210 were visible on the TLC pictures (Figure 3), with concentrations 2.5 times higher than VFA in  
211 order to be able to visualize corresponding spots. Succinic acid gives the highest response  
212 but R<sub>f</sub> of its derivatives is much lower than VFA. Formic and lactic acid derivatives have  
213 similar R<sub>f</sub> to acetic acid, but intensity of the response is much lower. If we compare these  
214 carboxylic acids with acetic acids at the same concentration, intensity response obtained with  
215 densitometric histograms is <4% of that of acetic acid. Other carboxylic acids tested (tartaric,  
216 oxalic, malic, maleic, citric, pyruvic, benzoic acid) led to no appreciable response even at 500  
217 mg.L<sup>-1</sup>. Considering the expected prevalence of VFA in real samples analyzed by this  
218 method, it is reasonable to conclude that VFA measurements with this TLC protocol should  
219 be free of interference from other compounds.

220

### 221 **3.4 Analytical features**

222 Analytical features of the method are summarized in Table 2, with TLC picture and  
223 associated densitometric histograms of a VFA calibration range between 10 and 125 mg.L<sup>-1</sup>  
224 represented on Figure 4 (left part). Polynomial regression equations were obtained, probably  
225 due to the visualization and detection method. Calibration ranges can be easily modified  
226 (increase of the lowest and highest bound of the calibration range) by reducing sample  
227 volume, depending on real samples analyzed. For example, reducing sample volume to 25  
228 µL enables an extension of the calibration ranges above 1 g.L<sup>-1</sup>, which will be more adapted  
229 to VFA concentrations in sewage sludge samples. Detection limits and quantification limits  
230 have been evaluated based on repetitions (n=7) of a sample spiked at 7 mg.L<sup>-1</sup> of each VFA,  
231 following U.S. EPA guidelines [25]. Detection limits and quantification limits were between  
232 1.5-2.5 mg.L<sup>-1</sup> and 4.7-7.9 mg.L<sup>-1</sup>, respectively. These limits were clearly low enough for our  
233 analytical purposes and were in the same range as those obtained by the GC-FID method  
234 used in our laboratory. Finally, relative standard deviation (RSD) between 4.6 and 6.7 %  
235 were obtained after application of the whole protocol on a 50 mg.L<sup>-1</sup> standard (n=7, with one  
236 TLC plate for each repetition). Precision with our method is therefore lower than that usually  
237 obtained with TLC scanner and dedicated data analysis software [18, 26], but is satisfactory  
238 enough for our analytical purpose using low-cost instruments with easy to find materials and  
239 software.

240

### 241 **3.5 Analytical costs**

242 The aim of the method described in this article is to provide a low-cost analytical tool to  
243 operational laboratories for which GC or HPLC is too expensive, both as investment and  
244 routine usage. Investment for specific equipment for our method is very low, as only standard  
245 vials, glassware and pipettes are necessary. Operational costs are summarized in Table 3  
246 and each sample analysis costs globally only 0.12 €, which makes this method indeed very  
247 affordable

248

### 249 **3.6 Comparison with other TLC method for VFA determination**

250 Direct TLC detection of VFA (without derivatization) is of course possible but detection limits  
251 are obviously too high (g.L<sup>-1</sup> range [27]) for our analytical application. Derivatization prior to  
252 TLC separation and detection is therefore mandatory to reach mg.L<sup>-1</sup> detection range. In  
253 spite of the clear advantages that TLC techniques could provide for VFA determination  
254 (simplicity, no sample clean-up and cross contaminations, cost), only one method has been  
255 published in the literature for derivatization of VFA and ppm detection. This derivatization  
256 method is very tedious and time-consuming, as it requires neutralization of the sample,



257 evaporation to dryness, boiling for 1 h with derivatization reagent, extraction with chloroform  
258 and evaporation to dryness of organic extracts [28]. In comparison, our method only requires  
259 reaction at room temperature for 6 min with non-toxic reagents and direct spotting of the  
260 mixture without evaporation (analytical step which has to be avoided for simplicity reasons  
261 for routine analysis as described in this study).

262 Therefore, no TLC method suitable for easy and practically applicable determination of  
263 individual VFA were described so far in the literature. Regarding our method, staff effort is  
264 moderate, as it takes only about 20 minutes to perform the whole analytical protocol (from  
265 derivatization to TLC final picture) for 3 samples (or 6 if no duplicates is performed), and  
266 technicity required for the routine application of the protocol is quite low, as no complex  
267 instruments are needed. This method is therefore well suited in laboratories where the global  
268 operational needs and costs for analytical tools are decisive for the routine and precise  
269 monitoring of their process.

270

### 271 **3.7 Validation on real samples**

272 A comparative study between our TLC method and GC-FID analysis has been conducted on  
273 real samples. This validation study was performed on a set of aqueous extracts of composts  
274 (made from anaerobically digested sewage sludge and green wastes) which were analyzed  
275 in our lab for their VFA content in the frame of a research project dealing with compost  
276 monitoring and early warning parameters (VFA was one of the studied parameters, as  
277 considerable variation of VFA has already been demonstrated in composting platform [29]).  
278 TLC picture and densitometric histograms used for quantification are shown on Figure 4  
279 (right part) for comparison with the calibration range used (scales for the left and right  
280 histograms are different and peak heights can't be compared visually). As expected, it can be  
281 observed visually that acetic acid is the prevalent VFA in the compost extracts with a  
282 noticeable evolution of VFA during the composting process (increase from AN1 to AN3  
283 during bio-oxidation phase and decrease for older composts during maturation phase).  
284 Quantitative results for individual C2–C5 VFA concentrations measured by our method or by  
285 GC-FID are detailed in Table 4. Results between both methods are in good agreement, with  
286 only slight underestimation by our TLC method. Standard deviations are also higher for the  
287 TLC method, but results are globally very satisfactory if we consider the simplicity of the  
288 material used for visualization and quantification. Note that semi-quantitative measurements  
289 could be possible by simple naked-eye comparison with the standard TLC plates for  
290 operational laboratories which just need early warning of a sudden change in VFA  
291 concentration or composition.

292

## 293 **4. Conclusions**

294 We have demonstrated that individual VFA determination and quantification using one of the  
295 simplest separation technique (thin-layer chromatography) is indeed possible for  
296 environmental analysis. Derivatization of VFA using a simple and rapid procedure at room  
297 temperature leads to the formation of derivatives which can be easily separated on silica gel  
298 TLC plates and detected without heating by simple dipping in a visualization solution. Use of  
299 a home-made black box enables reproducible smartphone pictures for subsequent  
300 quantification with a free software. As investment and operational costs are very low, this  
301 analytical method is perfectly suited for operational laboratories which can't afford on-site  
302 HPLC or GC analysis but require routine, rapid and low-cost measurement of individual VFA.  
303 The proposed method could therefore supplement classical titrimetric methods which only  
304 gives access to total VFA with no details on VFA composition, although this parameter is  
305 more and more recognized for its meaningful significance for the monitoring of anaerobic  
306 digestion processes.

307

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#### 424 **Figure captions**

425 **Figure 1.** Visualization of spots of VFA derivatives with PMA at 3% in EtOH (A) and PMA at  
426 3% in water (B). All VFA at 100 mg.L<sup>-1</sup>, mobile phase: ethyl acetate/cyclohexane: 60:40, v/v.  
427 Pictures taken out of the box in preliminary experiments.

428 **Figure 2.** Influence of mobile phase composition (ethyl acetate/cyclohexane, volume  
429 composition) on the separation of VFA derivatives (acetic, propionic, butyric and valeric acid  
430 at 100 mg.L<sup>-1</sup>). A = Acetic acid; P = Propionic acid; B = Butyric (+ Isobutyric) acid; V = Valeric  
431 (+ Isovaleric) acid.

432 **Figure 3.** Visualization of potential interfering compounds after application of the analytical  
433 protocol. C = Citric acid (250 mg.L<sup>-1</sup>); F = Formic acid (250 mg.L<sup>-1</sup>); G = Glycine (250 mg.L<sup>-1</sup>);  
434 VFA = mixture of all VFA at 100 mg.L<sup>-1</sup>; L = Lactic acid (250 mg.L<sup>-1</sup>); S = Succinic acid (250  
435 mg.L<sup>-1</sup>).

436 **Figure 4.** TLC picture of a calibration range (10-125 mg.L<sup>-1</sup>) with associated densitometric  
437 histogram (left part); TLC picture of real samples with associated densitometric histogram  
438 (right part). Scales for the two histograms are different (optimized by the software on the  
439 highest peak).



441 **Tables**

442 Table 1. Resolution between VFA peaks on densitometric histograms (with each VFA at  
 443 100mg.L<sup>-1</sup>), depending on the mobile phase composition.

Mobile phase composition (Ethyl acetate/ Cyclohexane, v/v)	50:50	60:40	70:30	80:20
$R_{S(V/B)}^a$	0.88	1.03	0.88	0.81
$R_{S(B/P)}^b$	1.21	1.32	1.15	1.23
$R_{S(P/A)}^c$	1.78	2.18	2.02	2.22

- 444 a. Resolution between valeric and butyric acid peak.  
 445 b. Resolution between butyric and propionic acid peak.  
 446 c. Resolution between propionic and acetic acid peak.

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Table 2. Analytical features of the proposed method

Parameter	Acetic acid	Propionic acid	Butyric acid	Valeric acid
Rf	0.24	0.43	0.55	0.64
Calibration range (mg.L <sup>-1</sup> )	7-125	6-125	5-125	8-125
Polynomial regression equation	$y = -1.72x^2 + 638.5x + 523.7$	$y = -2.38x^2 + 756.8x - 1812.1$	$y = -2.26x^2 + 862.8x - 1063.8$	$y = -1.47x^2 + 721.7x + 167.8$
Correlation coefficient	0.999	0.998	0.998	0.998
Detection limit (mg.L <sup>-1</sup> )	2.1	2.0	1.5	2.5
Quantification limit (mg.L <sup>-1</sup> )	6.7	6.3	4.7	7.9
RSD (%) <sup>a</sup>	4.6	5.3	6.7	6.5

- 450 a. Repetition of a 50 mg.L<sup>-1</sup> standard (n=7) on 7 different TLC plates.

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452 Table 3. Operational costs of the analytical protocol described in section 2.3.

Reagent/material	Quantity	Price	Cost/analysis (€)
HOAT	1.8 mg	100€/25g	0.0072
EDAN	0.6 mg	34€/25g	0.000816
EDC	3.5 mg	50€/25g	0,007

KH <sub>2</sub> PO <sub>4</sub>	2.5 mg	40€/500g	0.0002
Cyclohexane	0.4 mL <sup>a</sup>	17€/L	0.00688
Ethyl acetate	0.6 mL <sup>a</sup>	8.5€/L	0.0051
Phosphomolybdic acid	3 g <sup>b</sup>	60€/100g	0.009
TLC plates (6x6 cm)	1/9 of a full plate (20x20 cm) <sup>a</sup>	35€/25 full plates	0,156
<i>Total for 3 samples with duplicate analysis (6 spots) for one TLC plate</i>			0.375
<b>Total/sample</b>			<b>0.125</b>

453 a. For one TLC plate (6 spots = 3 samples with duplicate analysis).

454 b. For 100 mL of PMA solution (usable for 200 TLC plate visualization).

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456 Table 4. Comparative results between TLC and gas chromatography (GC) analysis on  
457 various compost extract samples (mg.L<sup>-1</sup>, mean ± standard deviation, n=2 replicates).

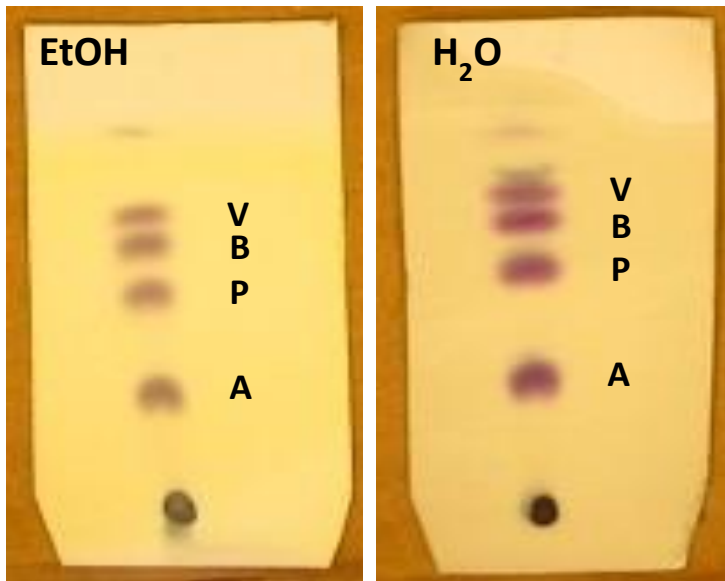
	C2		C3		C4		C5	
	GC	TLC	GC	TLC	GC	TLC	GC	TLC
AN1	68.7±2.5	63.8±3.2	9.5±1.9	7.1±2.1	16.2±1.0	14.8±4.1	15.3±0.2	12.0±1.2
AN2	80.2±1.7	88.7±10.1	11.0±0.6	8.3±1.3	19.0±1.4	17.2±0.9	18.6±0.1	14.4±0.8
AN3	131.1±5.4	121.2±5.6	14.7±2.2	10.0±2.4	23.3±0.7	18.3±3.8	14.9±0.6	12.9±1.4
AN4	41.6±4.7	36.4±2.3	12.2±0.5	11.4±1.3	13.2±1.2	9.5±2.0	3.7±0.2	<LQ
AN5	36.9±0.3	29.8±6.8	5.4±0.1	<LQ	7.9±0.3	5.3±1.0	5.2±0.5	<LQ

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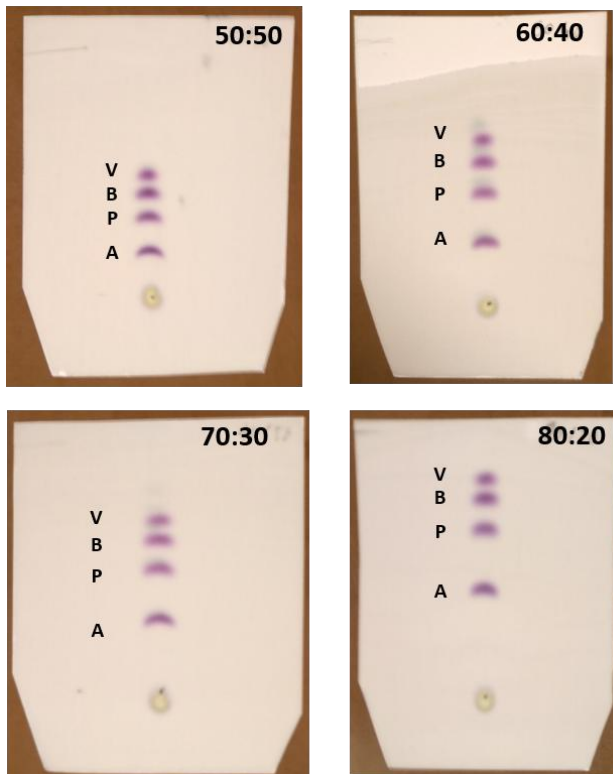


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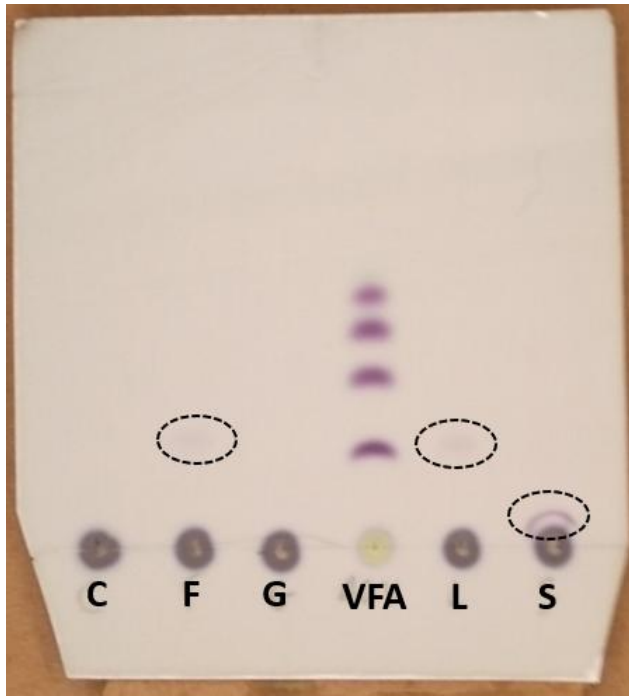


Figure 3

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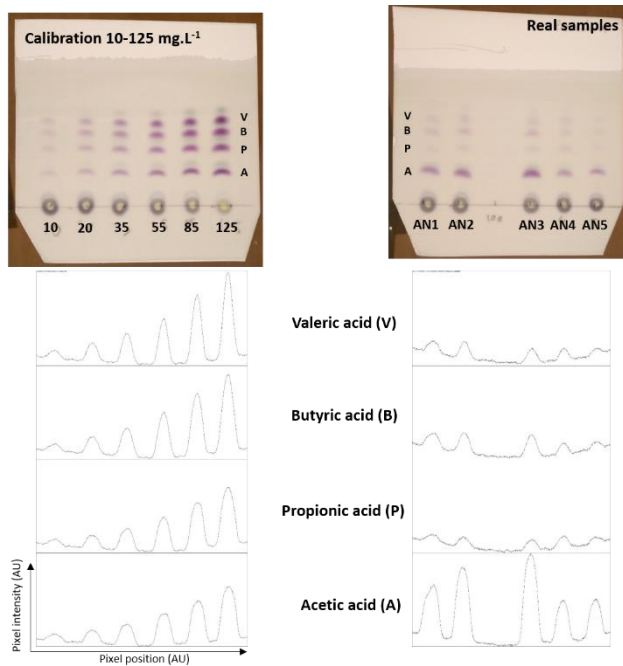


Figure 4

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