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Published on: 04 Apr 2019 - Analytical Methods (The Royal Society of Chemistry)

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F. Robert-Peillard, Elodie Mattio, Ainhoa Komino, Jean-Luc Boudenne, Bruno Coulomb. Development of a simple, low-cost and rapid thin-layer chromatography method for the determination of individual volatile fatty acids. *Analytical Methods*, Royal Society of Chemistry, 2019, 10.1039/c9ay00158a . hal-02090448

HAL Id: hal-02090448

<https://hal.archives-ouvertes.fr/hal-02090448>

Submitted on 4 Apr 2019

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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 ($1.5\text{-}2.5\text{ mg}\cdot\text{L}^{-1}$) 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 C₁₈ 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⁻¹ 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⁻¹
92 and the derivatizing reagent N-(1-naphthyl)ethylenediamine (EDAN) (Sigma-Aldrich) at 2 g.L⁻¹
93 ¹, prepared as a mixed solution in ultrapure water (Millipore, resistivity >18 MΩ cm) with final
94 pH set at 3.6. “KH₂PO₄ solution” was made of potassium dihydrogen phosphate (KH₂PO₄)
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⁻¹ for acetic acid, 1500 mg L⁻¹ 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 μm 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 μL of "HOAT+EDAN solution", 250 μL of sample and 40
114 μL of "EDC solution". The vial was stirred and left to react for 5 minutes. 75 μL of " KH_2PO_4
115 solution" were then added and the vial was again left to react for 1 minute after proper
116 stirring. 3 μL of the reaction mixture was then manually spotted on the TLC plate using 3 μL
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 μm 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 mg L^{-1} (valeric acid) and 10 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 g.L^{-1} for EDC and 2 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⁻¹ (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_f 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_f of its derivatives is much lower than VFA. Formic and lactic acid derivatives have
213 similar R_f 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⁻¹. 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⁻¹
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⁻¹, 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⁻¹ of each VFA,
231 following U.S. EPA guidelines [25]. Detection limits and quantification limits were between
232 1.5-2.5 mg.L⁻¹ and 4.7-7.9 mg.L⁻¹, 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⁻¹ 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⁻¹ range [27]) for our analytical application. Derivatization prior to
252 TLC separation and detection is therefore mandatory to reach mg.L⁻¹ 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

308 **Acknowledgment**

309 This work was financially supported by the French Environment and Energy Management
310 Agency (ADEME) through the programme "ODIAMAC" [Convention n°1506C0034].

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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⁻¹, 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⁻¹). 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⁻¹); F = Formic acid (250 mg.L⁻¹); G = Glycine (250 mg.L⁻¹);
434 VFA = mixture of all VFA at 100 mg.L⁻¹; L = Lactic acid (250 mg.L⁻¹); S = Succinic acid (250
435 mg.L⁻¹).

436 **Figure 4.** TLC picture of a calibration range (10-125 mg.L⁻¹) 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⁻¹), 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 ⁻¹)	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 ⁻¹)	2.1	2.0	1.5	2.5
Quantification limit (mg.L ⁻¹)	6.7	6.3	4.7	7.9
RSD (%) ^a	4.6	5.3	6.7	6.5

- 450 a. Repetition of a 50 mg.L⁻¹ 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 ₂ PO ₄	2.5 mg	40€/500g	0.0002
Cyclohexane	0.4 mL ^a	17€/L	0.00688
Ethyl acetate	0.6 mL ^a	8.5€/L	0.0051
Phosphomolybdic acid	3 g ^b	60€/100g	0.009
TLC plates (6x6 cm)	1/9 of a full plate (20x20 cm) ^a	35€/25 full plates	0,156
<i>Total for 3 samples with duplicate analysis (6 spots) for one TLC plate</i>			0.375
Total/sample			0.125

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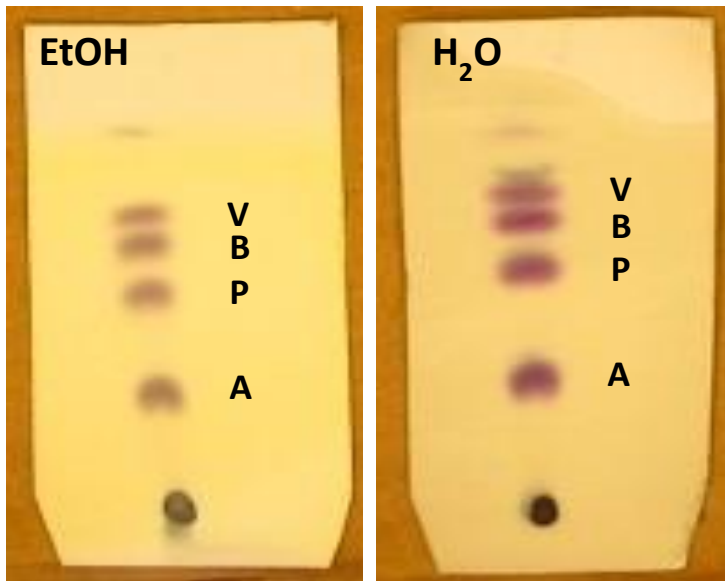
456 Table 4. Comparative results between TLC and gas chromatography (GC) analysis on
457 various compost extract samples (mg.L⁻¹, mean ± standard deviation, n=2 replicates).

	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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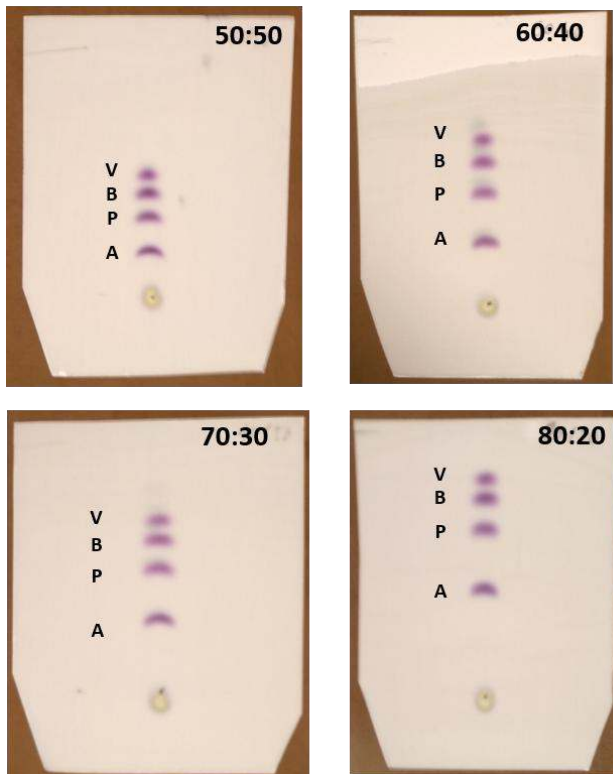
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469 Figure 1

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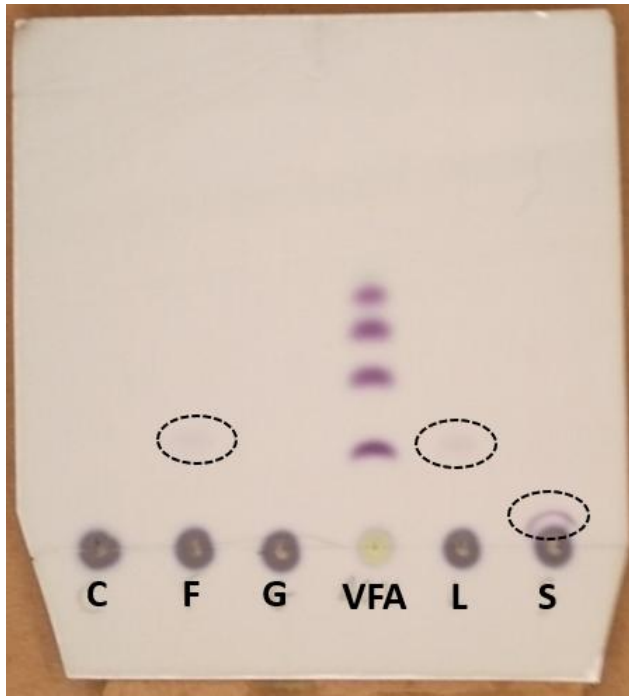
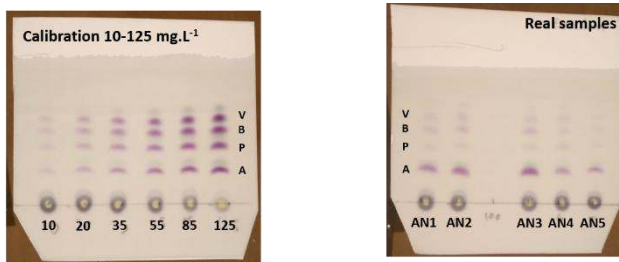
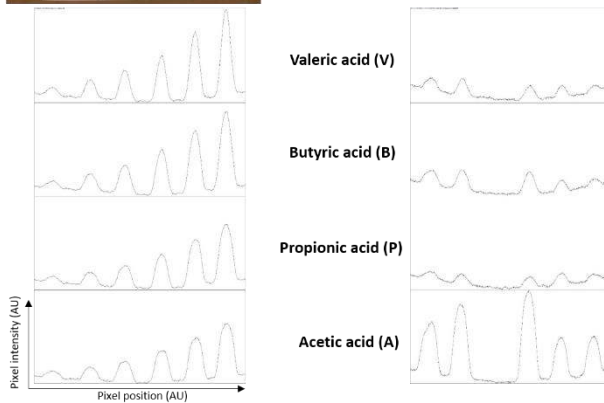


Figure 3

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Figure 4

