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Towards in field miniaturized liquid chromatography: Biocides in wastewater as a proof of concept

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ABSTRACT

Solving and/or evaluating given problems or decision making in place and in real time is a goal of the analytical chemistry science. In this context, the performance of a commercial portable miniaturized liquid chromatograph (minLC) with LED UV (255 nm) detector was compared with those provided by two lab minLCs (capillary and nano) coupled on-line to in-valve in-tube solid phase microextraction (IT-SPME) with diode array detector (DAD). In addition, responses of the portable LC for in-field analysis in several conditions were tested. Besides, two evaluation tools, BETTER criteria for portability and HEXAGON pictogram for sustainability and greenness were applied for comparison purposes. The benchtop LCs provided lower limits of detection (LODs) as expected, in the order of low μg L^{-1}, than those achieved by the portable LC, with LODs around mg L^{-1} for compounds covering several polarities (logKow between -1.72 and 3.82). The used portable LC gave excellent resolution, reducing the analysis time and being the consumption of solvents negligible. As a practical application, fruit washing residual waters, which contained a suitable level of concentrations of several biocides for employing the portable minLC, were analyzed and quantified from the three minLCs as a proof of concept with comparable results.

1. Introduction

Miniaturized liquid chromatography (minLC) emerged as a consequence of the reduction of the column internal diameter, which comes along with a reduction in mobile phase flow rates [1–3]. LC can be classified according to the analytical column size, particularly inner diameter (i.d.) in conjunction or not with flow rates. Nomenclature used by Chervet et al. [2] is shown in Table S1 of the supporting information (SI).

Portable LC evolved slowly compared to LC in general [3]. This is most likely due to the difficulties encountered in miniaturizing the high-pressure pumping system and detectors while maintaining an acceptable robustness and performance [4]. Since the first portable LC system was reported in 1986 [5], several other instruments were developed [6–14] as it can be reported in Table 1. Rahimi et al. [15] in 2020 compared several portable LC systems [5–11,13,14] using the BETTER (portaBle field Testing sTandard framEwoRk) criteria, which acts as a framework to facilitate comparison between the different instruments. Rahimi et al. concluded that despite the fact that each of them brings different improvements over the chromatographic separation, there are several areas where more attention is required, such as to offer sufficient performance in a cost-competitive manner and to ensure robustness and stability in the field [15].

Almost all these portable LCs were homemade and were not commercialized (Table 1) and there is only one published work for the most of them. The MiLiChrom-4 (Microcolumn Liquid Chromatographs) was one of the few commercialized portable-LC. There are several reports showing its capabilities in different areas, but its high weight (14 kg) and the fact of not being battery operated makes it difficult to truly consider it as portable. Abonamah et al. [12] utilized a commercial nanoLC (Easy NanoLC Thermo) coupled to EI-MS detector for on-site detection of fentanyl and its derivatives. As for the previous LC, its higher weight, 37 kg, makes hard to consider it as portable. There are several works where Easy Nano-LC is utilized for different determinations, however, most of them use it as a benchtop nano-LC.

Recently, the Axcend Focus LC (weight 8 kg), a new fully portable minLC has been commercialized. There are some works where Axcend Focus LC is used, specifically for the determination of drugs [16,17], trimethylxanthines in waters [18,19], scopolamine in beverages [20] and for common educational demonstrations in-

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Table 1
Portable-LC systems developed to date.

Reference	Year	Commercialization	Column i.d.	Column type	Published Works	Area of work
[5]	1986	Non-commercial	4.6 mm	Conventional	1	PAAs
MiLiChrom-4 [6]	1996	Commercial	2 mm	Microbore	7	Drugs, pesticides, PAHs, phenols, phthalate esters and inorganic analytes
Minichrom [7]	1998	Non-commercial	4.6 mm	Conventional	1	Phenols in water
[8]	2015	Non-commercial	0.8 mm	Micro	1	Phenols, catecholamines, catechin and amino acids
[9]	2015	Non-commercial	150 µm	Nano	1	Pesticides and phenols.
[10]	2015	Non-commercial	100 µm	Nano	1	Parabens
[11]	2017	Non-commercial	100 µm	Nano	1	Peptide and protein mixes
Easy nano-LC (Thermo) [12]	2919	Commercial	300 µm	Capillary	1[a]	Fentanyl and derivates
[13]	2020	Non-commercial	150 µm	Nano	1	Amino acids and fungicidal pesticides
[14]	2021	Non-commercial	75 µm	Nano	1	Pharmaceutical compounds
Axcend Focus LC (used in this work) [16–21]	2020–2021	Commercial	150 µm	Capillary Nano	7	Trimethylxanthines, scopolamine, and pharmaceutical, illicit, and emerging drugs
PAHs portable LC [22]	2021	Non-commercial	2.1 mm	Microbore	1	PAHs

a There is more than one work but only one where the Easy nano-LC is used as a portable.

volving separations [21]. In 2021 Chatzimichail et al. [22] developed a new portable LC with UV-vis spectral detector using a microbore column for determining PAHs in waters and BETTER criteria was evaluated for it.

In this work, we used a commercial portable minLC in order to study the achievements and needs in reference to benchtop minLC, both capillary and nano, for contributing towards in place minLC. BETTER criteria [15] for portability and also HEXAGON tool [23,24] for evaluating greenness and sustainability of lab and portable instruments were applied. Compounds covering several polarities ($\log K_{ow}$ between −1.72 and 3.82) were selected (Table S2 of SI) for testing capacities and real fruit washing waters were analyzed as a proof of concept.

2. Experimental section

2.1. Reagents and solutions

All reagents were of analytical grade. HPLC grade acetonitrile and methanol were supplied by VWR chemicals (Radnor, PA. USA). Acetaminophen (AMP), caffeine (CAF), dimethyl phthalate (DMP), metribuzin (MBZ), isoproturon (IPT), imazalil (IMZ), o-phenyl phenol (OPP), potassium sorbate (PS), proxyphylline (PPL), pyrimethanil (PMA), theobromine (TBM) and theophylline (TL) were obtained from Sigma-Aldrich (St. Louis, MO. USA). Fluometuron (FMT) was obtained from Dr. Ehrentorfer (Augsburg, Germany). The stock solutions (in the range of 1000–4000 µg L^{-1}) of the individual analytes were prepared in ultrapure water from an Adrona system (Riga, Latvia) (PS), methanol (CAF, PPL and TPL) or acetonitrile (AMP, TBN-m, DMP, MBZ, FMT, IPT, OPP, PMA and IMZ) and stored at 4 °C. Working solutions were prepared in ultrapure water by dilution of the stock solutions. All the solutions were filtered through a 0.22 µm nylon membrane filter supplied by GVS (Dr. Sandford, ME, USA).

2.2. Equipment and chromatographic conditions

The analysis was performed using a portable minLC (Axcend Focus LC, Provo, UT. USA). The system consists of two high-pressure syringe pumps capable of delivering flow rates in the nano and capillary scale (0.5–10 µL min^{-1}, see Table S1 of the SI) at pressures up to 410 bar, connected to a mixing valve (capable of work in either isocratic or gradient mode) so a single mobile phase reaches the injection valve with and internal loop of 40 nL. A column (100 × 0.150 mm i.d.) packed with ODS of 1.7 µm particle size is mounted inside a cartridge, which also contains an on capillary UV absorbance detector with a light emitting diode (LED) with a working wavelength of 255 nm. The samples were loaded in the injection port by means of a 25 µL Hamilton syringe. Data were acquired and processed with the Axcend Focus v2 software. For the separation of the analytes a mixture of solvent A (97:3 H_2O:ACN) and B (3:97 H_2O:ACN) in gradient elution was used as mobile phase. The elution gradient started at 5% of B and was increased to 20, 60, 95% of B at 4.0, 4.5 (held 1 min), and 6.0 min (held 0.5 min). The mobile phase flow rate and equilibration time were 2.0 µL/min and 2 min, respectively.

A lab capillary LC (Agilent Infinity 1260) adapted for use in valve in-tube solid phase microextraction (IT-SPME) with a binary pump that allows to work with flows lower than 20 µL min^{-1} and a 6-way injection valve (Rheodyne 7721-I, IDEX Health and Science), was used. The open IT-SPME capillary consisted in a 20 cm-segment of 0.32 mm i.d. untreated fused silica capillary (Análisis Vínicos, Tomelloso, Spain) with a volume of 16 µL, which was coated with tetramethylorthosilicate (TEOS), trimethoxymethylsilane (MTEOS) and SiO_2 nanoparticles (NPs) with a thickness of 350 nm following the procedure described by Serra-Mora et al. [25]. ZORBAX SB-C18 (150 × 0.5 mm, 5 µm and 35 × 0.5, 5 µm) columns from Agilent Technologies (Waldbronn, Germany) were used for chromatographic separation. The signal was recorded between 200 and 400 nm and monitored at 220, 250 and 270 nm with a diode array detector (DAD) equipped with a 80 nL nanoflow cell (Agilent Technologies). The detector was coupled to a data system (Agilent, ChemStation) for data acquisition and treatment. The gradient used was: percentage of ACN in the mobile phase started at 7% and was increased to 10, 45 (kept 2 min.), 70 (kept 2 min.), 80 (kept 2 min.) and 95% (kept 3 min.) at 1, 2, 5, 8 and 11 min., respectively. The flow rate was 10 µL min^{-1} and the processed sample volume of 50 µL.

For the lab nanoLC assays an Agilent 1200 Series (Agilent) coupled on line to IT-SPME was used by in-valve mode. The capillary used for IT-SPME consisted in a 10 cm-segment of 75 µm i.d. untreated fused silica capillary (Análisis Vínicos) with a volume of 440 nL, which was coated with TEOS-MTEOS-SiO_2 with a thickness of 350 nm [25]. The chromatograph was equipped with a Rheodyne 7725i 6 ports – 2 positions, 1/16″ and an automatic VICI C2N 10 ports – 2 positions, 1/32″ and 100 µm port size injection valve and a DAD detector (Agilent). Two different columns were used to carry out the separations, 50 × 0.075 mm, 3.5 µm and 150 × 0.1 mm, 3.5 µm Zorbax 300SB-C18 (Agilent). Data were acquired and processed by Agilent HPLC ChemStation Software. Signal was recorded in the range of 200–400 nm and monitored at 220, 250 and 270 nm. As mobile phase a mixture of H_2O and ACN was used in gradient elution mode. The elution program started

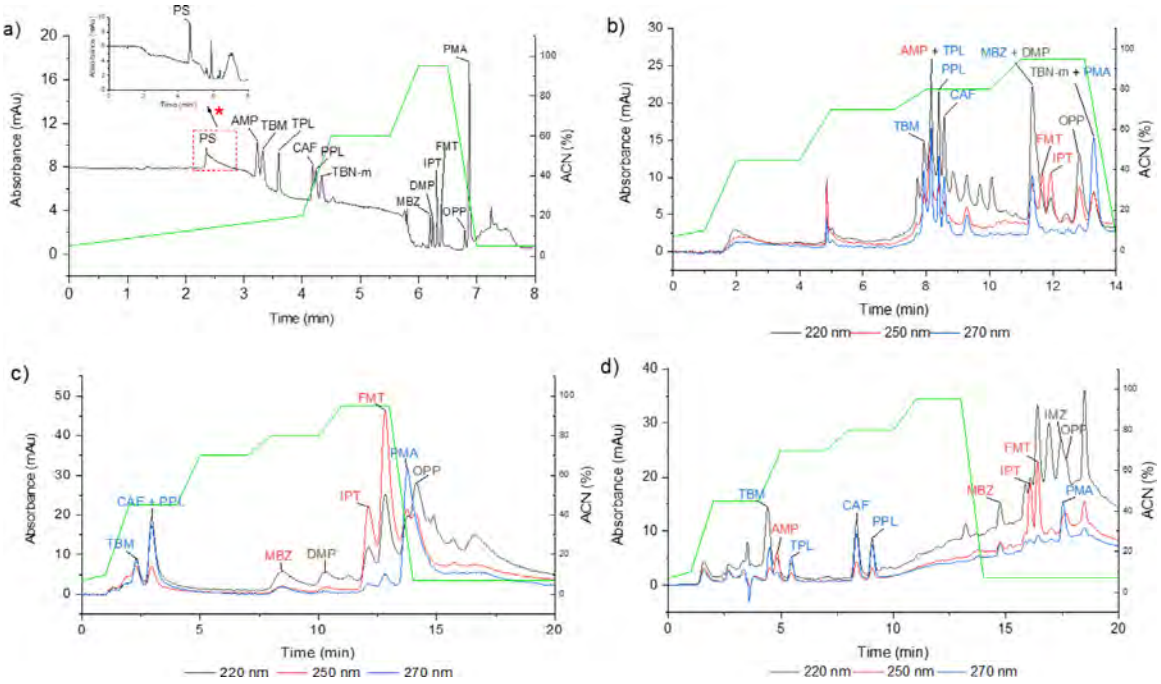

Fig. 1. Chromatograms for the multicomponent mixtures with the portable Nano-LC at 255 nm (a), the benchtop CapLC (b), and the benchtop NanoLC with 50 × 0.075 mm (c) and 150 × 0.1 mm columns (d) were done at three wavelength. For chromatogram (a), the asterisk (*) shows the PS peak obtained carrying the analysis with ACN and water with 0.1% of formic acid as mobile phase solvents. See Table S3 of the SI for tested concentrations and experimental section for more explanations.

at 7% of ACN and was increased to 10, 45, 70, 80 and 95% ACN at 1, 2 (held 2 min), 5 (held 2 min), 8 (held 2 min) and 11 min (held 2 min), respectively. The mobile phase flow rate was 0.5 µL min $^{-1}$. The injection system consisted of a first 6 port – 2 positions injection valve where the sample is injected and transferred to the second 10 port – 2 positions valve by means of a untreated fused silica capillary (15 × 0.1 mm, 800 nL) and passing through the SPME capillary, being the processed sample volume of 50 µL. Switching the second valve positions the sample was eluted by the mobile phase to the analytical column. Fig. S1of the SI shows a schematic representation of the injection system.

2.3. Multicomponent mixtures and samples

For multicomponent mixtures the tested concentrations are given in Table S3 of the SI.

Samples from three different washing treatments containing pesticides for plague control realized in three different places were analyzed. Samples were filtered through a 0.22 µm nylon filter (Labbox, Barcelona, Spain) and diluted 1:100 in ultrapure water for injection in portable LC (for the separation of potassium sorbate solvents A and B of the mobile phase contained 0.1% of formic acid) and 1:5000 and 1:10,000 for the benchtop capLC (short column) and nanoLC (short and long columns), respectively. All samples were stored at 4 °C. Pesticides used for the treatment were imazalil and potassium sorbate in the sample 1, and imazalil and pyrimethanil in the sample 2 and sample 3.

3. Results and discussion

3.1. Chromatographic performance

Different chromatographic conditions were tested to separate the analytes for the three different LCs. For portable LC the components of the mobile phase and the gradient conditions were optimized. For benchtop cap and nano LCs besides gradient conditions, size and phase of the IT-SPME capillaries were tested. The optimized conditions are given in Sections 2.2 and 2.3. Chromatograms obtained for the several multicomponent solutions (see Table S3 of the SI) for both, portable, and benchtop cap and nano LCs, are shown in Fig. 1. We selected segmented gradients, which reduced the separation time and improved resolution. This figure includes the optimum gradient used for each chromatography and for capLC and nanoLC the several wavelengths used for quantitation (220, 250 and 270 nm in accordance with the absorption maxima of the tested compounds). Log K_{OW} of the compounds assayed are given in Table S2 of the SI. Fig. 1a shows a very good mixture resolution achieved by the portable minLC, which only works at 255 nm by LED detection. The resolution achieved by the benchtop capLC (see Fig. 1b) was worse than that obtained by the portable minLC; several analytes provided overlapped chromatographic peaks. On the other hand, as Fig. 1c shows, working with the 50 × 0.075 mm column, the benchtop nanoLC gives lower performance than that corresponding to that obtained by the column with 150 × 0.1 mm dimensions. This means that this last column provided better performance of the nanoLC system. The time window for the resolution of the assayed mixtures were different for the three systems as Fig. 1 shows: 8, 14 and 20 min for portable minLC, benchtop capLC and nanoLC with the longer column, respectively. CapLC resolved worse mixture separation as mentioned and the chromatographic profiles obtained by portable minLC and benchtop nanoLC were similar, although the overall resolution of the portable LC was greater than that provided by the benchtop nanoLC. This fact can be explained mainly by the particle sizes of the different columns employed (1.7 µm vs 3.5 µm, for portable and nanoLC, respectively), which are limited by the pressure achieved by the several assayed LC systems. Table 2 gives peak widths calculated from the software of each equipment as a measure of column performance in gradient elution. Achieved precision of the retention times for all LC systems and analytes were lower than 1%. The mean peak widths and their standard deviations obtained from data given in Table 2 are: 0.07 ± 0.01, 0.14 ± 0.05 and 0.18 ± 0.03 min for

Table 2
Peak widths at 4σ ($\bar{\omega}$) obtained for the multicomponent mixtures by the software of the LC instruments used. See Table S3 of the SI for tested concentrations and experimental section for more explanations.

	Portable LC 100 × 0.15 mm, 1.7 µm Loop volume = 40 nL		Benchtop Cap-LC 150 × 0.5 mm, 5 µm Loop volume = 16 µL		Benchtop Nano-LC 150 × 0.1 mm, 3.5 µm Loop volume = 440 nL	
Compound	t_r, min	$\bar{\omega}$, min	t_r, min	$\bar{\omega}$, min	t_r, min	$\bar{\omega}$, min
Potassium Sorbate	2.35	–	4.60	–	3.10	0.24
Theobromine	3.32	0.10	7.93	0.11	4.54	0.20
Acetaminophen	3.13	0.10	8.11	0.08	4.79	0.20
Theophylline	3.51	0.08	8.15	–	5.47	0.21
Caffein	4.18	0.07	8.57	0.09	9.86	0.20
Proxyphylline	4.24	0.07	8.40	0.09	8.91	0.20
Tribenuron-methyl	4.30	0.08	13.30	–	–	–
Metribuzin	6.20	0.06	11.35	0.14	15.32	0.18
Dimethyl Phthalate	6.25	0.06	11.46	–	15.00	0.12
Isoproturon	6.32	0.06	11.93	0.16	15.87	0.16
Fluometuron	6.39	0.06	11.66	0.14	16.01	0.17
o-phenyl phenol	6.80	0.07	12.84	0.21	14.73	0.14
Pyrimethanil	6.86	0.07	13.30	0.22	16.40	0.18

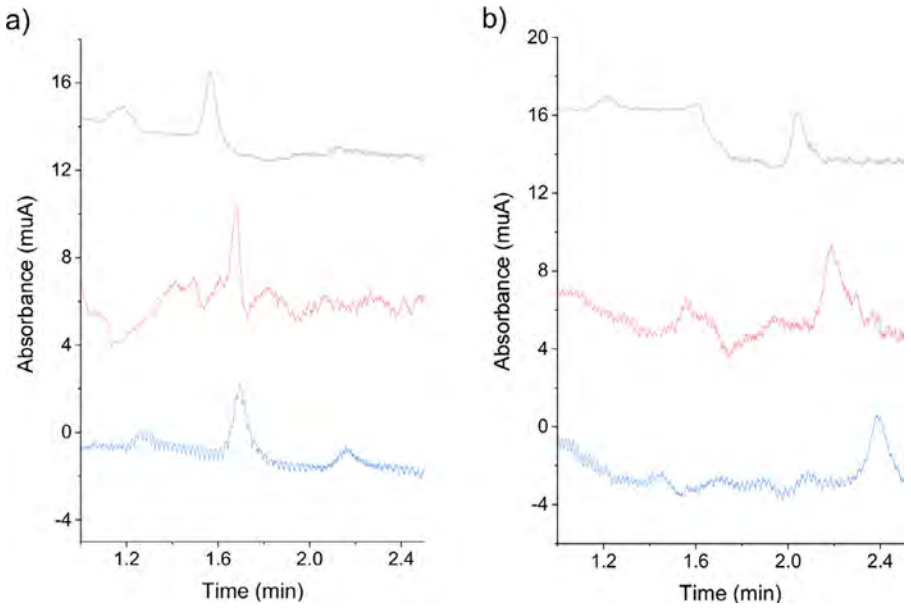

Fig. 2. Chromatograms obtained for (a) theobromine and (b) theophylline at different number of previous injections in the cartridge.

portable LC and benchtop capLC and nanoLC, respectively. Peak capacity (P_c) is a measure of the separation power that includes the entire chromatographic space together with the variability of the peak width over the chromatogram. If the peak width pattern over a chromatogram is very similar, as it is in most reversed-phase gradient separations and here, it can be calculated from Eq. (1) as described by U.D. Neue [26]:

$$P_c = 1 + \frac{t_G}{\bar{\omega}} \quad (1)$$

where $\bar{\omega}$ is the average peak width, and t_G is the gradient run time.

P_c can be used as a parameter for evaluating efficiency in gradient elution. The values calculated for portable LC and benchtop capLC and nanoLC were: 115, 101 and 112, respectively. The small differences can be explained by the dimensions of the analytical columns used and the particle sizes of the stationary phases (see Table 2). Note that the separation for the more polar compounds is achieved at higher water content for nano LC than for capLC.

The cartridge performance of the portable minLC was evaluated throughout the number of injections. Fig. 2 shows the chromatograms for 30 mg L^{-1} of a) theobromine and b) theophylline as targets at different number of previous injections (new, 700 and 1000 previous ones). Compared with the chromatogram obtained from a new cartridge, for both analytes at 700 and 1000 injections the peak profiles were similar.

3.2. Figures of merit

Different analytical parameters such as linearity in the working range, precision, sensitivity, and recovery were determined for the portable minLC and compared with the ones obtained for the benchtop capLC and nanoLC. Tables 3–5 show the results obtained. Limits of detection (LODs) were calculated as the concentration of analyte which gives a signal-to-noise ratio (s/n) equals 3. The limit of quantifications (LOQ) was the concentration at the lower working linearity level ($s/n = 10$). Accuracy obtained from standards is expressed as relative error (E_r).

Instrumental detection limits for the portable system are in the range of 0.4–10 mg L^{-1} as it can be seen in Table 3, allowing to determine analyte at trace levels (mg L^{-1}) but not at ultra-trace (µg L^{-1}) without any pre-concentration treatment. Benchtop cap LC and nanoLC provides as expected lower LODs, around three order of magnitude lower, between 0.5 and 5 µg L^{-1}. As Tables 4–5 shows. These tables also include the LODs achieved at 250 nm for showing the loss of detection in function of selected wavelength due to the portable LC only includes a single LED detector at

Table 3
Figures of merit obtained for determination of target analytes by means of a portable minLC. Regression line (y = (a ± sa) + (b ± sb) x), regression coefficient (R^2), working range, limit of detection (LOD), relative error (E_r) and recovery (R). * Solvents A and B of the mobile phase contained 0.1% of formic acid.

Compound	Working range (mg L^{-1})	a ± sa	b ± sb (L mg^{-1})	R^2	LOD (mg L^{-1})	E_r, %	R, % $n = 3$
Potassium Sorbate*	1.0–16	– 0.1 ± 0.1	1.584 ± 0.012	0.9999	0.4	–1	90 ± 10
Theobromine	3.0–22	0.48 ± 0.01	0.53 ± 0.09	0.9996	1	–2	
Acetaminophen	3.0–23	0.04 ± 0.3	0.58 ± 0.02	0.9970	1	–5	
Theophylline	1.8–12	0.2 ± 0.1	0.521 ± 0.014	0.9980	0.6	4	90 ± 10
Proxyphylline	3.0–10	0.33 ± 0.05	0.580 ± 0.008	0.9998	1	–1	
Caffein	3.0–24	0.3 ± 0.5	0.69 ± 0.03	0.9990	1	5	85 ± 7
Tribenuron-methyl	30–115	0.2 ± 0.3	0.075 ± 0.004	0.9940	10	1	
Isoproturon	2.0–20	– 0.3 ± 0.2	0.53 ± 0.02	0.9950	0.8	10	85 ± 10
Fluometuron	3.0–19	0.2 ± 0.3	0.42 ± 0.03	0.9900	1	–7	85 ± 10
Pyrimethanil	0.3–29	1.8 ± 1.8	3.32 ± 0.10	0.9980	0.1	–4	70 ± 8

Table 4
Figures of merit obtained for determination of target analytes by means of a benchtop CapLC. Regression line (y = (a ± sa) + (b ± sb) x), regression coefficient (R^2), working range, limit of detection (LOD), relative error (E_r) and recovery (R). * LOD estimated at 250 nm.

Compound/λ (nm)	Working range (mg L^{-1})	a ± sa	b ± sb (L mg^{-1})	R^2	LOD (μg L^{-1})	E_r %	R, % $n = 3$
Potassium Sorbate/ 250	15–500	60 ± 60	7.3 ± 0.2	0.990	5	2.0	95 ± 10
Theobromine/ 270	3.0–50	13 ± 3	2.60 ± 0.14	0.990	1, 5*	1.0	
Caffein/ 270	1.5–50	2.5 ± 1.6	3.38 ± 0.05	0.998	0.5, 2*	−1.0	95 ± 10
Theophylline/ 270	3.0–50	4.8 ± 1.6	2.36 ± 0.05	0.995	1, 5*	−4.5	120 ± 10
Acetaminophen/ 250	1.5–52	4 ± 2	3.90 ± 0.07	0.997	0.5	3.0	
Dimethyl Phthalate/ 220	5.0–50	0 ± 2	3.13 ± 0.07	0.995	2, 25*	2.0	
Metribuzin/ 270	5.0–50	2.4 ± 1.0	2.28 ± 0.03	0.998	2, 10*	−5.0	
Fluometuron/ 250	3.0–50	−0.5 ± 0.8	4.68 ± 0.03	0.997	1	6.0	110 ± 5
Isoproturon/ 250	3.0–50	−3.6 ± 2.0	4.53 ± 0.07	0.998	1	4.0	109 ± 9
Imazalil/ 220	15–1000	−70 ± 30	3.98 ± 0.07	0.998	5	−4.0	100 ± 5
Pyrimethanil/ 270	6.0–500	−110 ± 60	10.6 ± 0.02	0.998	2, 10*	−5.0	100 ± 5

Table 5
Figures of merit obtained for the determination of target analytes by means of a benchtop nanoLC with the longer column. Regression line (y = (a ± sa) + (b ± sb) x), regression coefficient (R^2), working range, limit of detection (LOD), relative error (E_r) and recovery (R). * LOD estimated at 250 nm.

Compound/λ (nm)	Working range (μg L^{-1})	a ± sa	b ± sb (L μg^{-1})	R^2	LOD (μg L^{-1})	E_r (%)	% Rec n = 3
Potassium Sorbate/ 250	15–60	1.6 ± 0.7	7.0 ± 0.3	0.9980	2	1.0	100 ± 5
Theobromine/ 270	15–60	1.6 ± 0.7	1.249 ± 0.016	0.9996	5, 12*	0.71	
Acetaminophen/ 250	8–60	−1.2 ± 1.9	1.76 ± 0.05	0.9980	2.5	−6.2	
Theophylline/ 270	3–60	0 ± 1	1.04 ± 0.03	0.9980	1, 4*	−4.4	120±10
Proxyphylline/ 270	3–60	4.3 ± 0.6	1.750 ± 0.015	0. 9994	1, 6*	−0.2	
Caffein/ 270	15–60	1.8 ± 0.6	2.541 ± 0.016	0.9999	0.5, 2*	1.3	120±10
Metribuzin/ 250	15–60	−0.9 ± 1.4	1.19 ± 0.04	0.9980	4	3.6	
Dimethyl Phthalate/ 220	9–60	−0.6 ± 1.4	0.89 ± 0.04	0.9970	3	2.5	
Isoproturon/ 250	3–60	14 ± 2	3.28 ± 0.06	0.9992	1	4.3	103±5
Fluometuron/ 250	3–60	6 ± 3	2.93 ± 0.01	0.9980	1	6.2	96±5
Imazalil/ 220	3–100	0.6 ± 1.5	1.20 ± 0.03	0.9990	1, 10*	−11.5	98 ± 5
o-phenyl phenol/220	6–60	8.5 ± 1.9	0.76 ± 0.04	0.9970	2, 12*	−3.3	
Pyrimethanil/270	6–80	46 ± 12	7.1 ± 0.3	0.9980	2, 10*	−7.6	100 ± 5

Table 6
Pesticide concentrations found in samples (mean ± IR 95%).

	Sample	Potassium Sorbate (mg L^{-1})	Imazalil (mg L^{-1})	Pyrimethanil (mg L^{-1})
Portable LC	M1	1800 ± 140	–	n.d.
	M2	n.d.	–	3.2 ± 0.3
	M3	n.d.	–	110 ± 20
Benchtop Nano LC	M1	1750 ± 20	150 ± 30	n.d.
	M2	n.d.	480 ± 20	3.6 ± 0.14
	M3	n.d.	450 ± 20	130 ± 20
Benchtop CapLC	M1	1800 ± 120	180 ± 30	n.d.
	M2	n.d.	480 ± 60	3.3 ± 0.2
	M3	n.d.	450 ± 20	120 ± 10

n.d.: not detected.

255 nm. Accuracy values for portable LC and benchtop capLC and nanoLC provided suitable values for environmental analysis (E_r < |15%|) [27].

Matrix effect was evaluated by spiking with several analytes (TPL, CAF, FMT, IPT) a well water, which contained the analytes below their LODs. The recovery values are shown in Tables 3–5. As it can be seen the obtained values for the three LC techniques are statistically similar. No matrix effect was present.

3.3. In-field analysis at several environmental conditions

The increasing need for in-situ analysis carried out in the field makes portable equipment indispensable. For a chromatograph to be used in-field analysis, not only do it needs to be battery-powered and lightweight, but it also need to provide reliable analytical results under adverse conditions. Therefore, the analytical performance of portable minLC was evaluated in the field un-

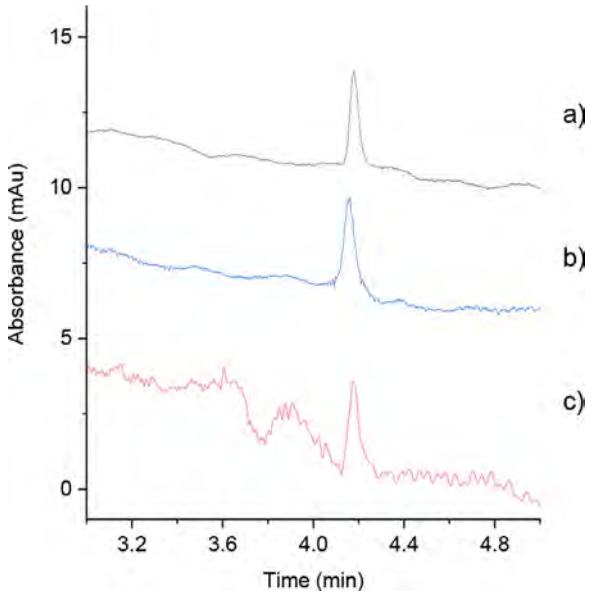

Fig. 3. Chromatograms obtained for caffeine at laboratory conditions (a) 22 °C and 45% humidity, field conditions (b) 14 °C, 68% humidity and 25 km/h wind (c) field conditions 7 °C, 73% humidity and 15 km/h wind.

der different conditions of humidity, wind, and temperature. We selected caffeine as target analyte for testing the performance. Fig. 3 shows the chromatograms obtained for CAF in laboratory and in-field conditions. Variation in humidity between measurements (45–80% RH) was not relevant. However, chromatograms baseline becomes somewhat noisier with the decrease in temperature. Wind speed did not modified the response. In all cases the chromatographic profile was maintained.

3.4. Sample analysis

Three residual waters from fruit washing treatment of three different places were analyzed. The waters can contain biocides like potassium sorbate (PS), imazalil (IMZ) or pyrimethanil (PMA). Figs. 4–6 show the obtained chromatograms, which were very clean in the interest time window. No matrix effect was obtained (see Tables 3–5). The sample 1 contained PS and IMZ, at levels of concentration given in Table 6. IMZ cannot be quantified by the portable minLC due to the instrument only have a LED of 255 nm. Then, from this instrument sample 2 and sample 3 shown in Figs. 5 and 6, respectively, only permitted to quantify PMA. Similar results were obtained from the three LC systems for PMA as it can be shown in Table 6, although the sample dilutions carried out for sample processing were very different. IMZ can be quantified from the benchtop instruments providing similar results.

3.5. Evaluation tools

3.5.1. BETTER criteria

To evaluate capacities of the Axcend Focus LC, the BETTER assessments defined by Rahimi et al. [15] were followed. BETTER grade levels (each ranging from 1 to 5) are selected both to represent the current range of developments, and community goals. To date, portable LC systems meet some of the grade 2 and 3 criteria [15,22]. The grade 4 and 5 criteria are deemed a challenge to be met by next generation devices; indeed, no instrument reported to date reaches Grade 5 in any category. Different non chromatographic and chromatographic characteristics (system cost, cost/test, weight, and performance) and other requirements for in-field analysis (portability, robustness, sample introduction) are considered.

The system cost of the Axcend Focus LC is in the average in relation to other portable chromatographic systems [15]. However, due to the low solvent consumption (∼ 16 µL per chromatographic run) the cost/test is markedly low. If we only consider the solvent usage, the cost/test is ∼ 0.0005 €. Assuming an average price

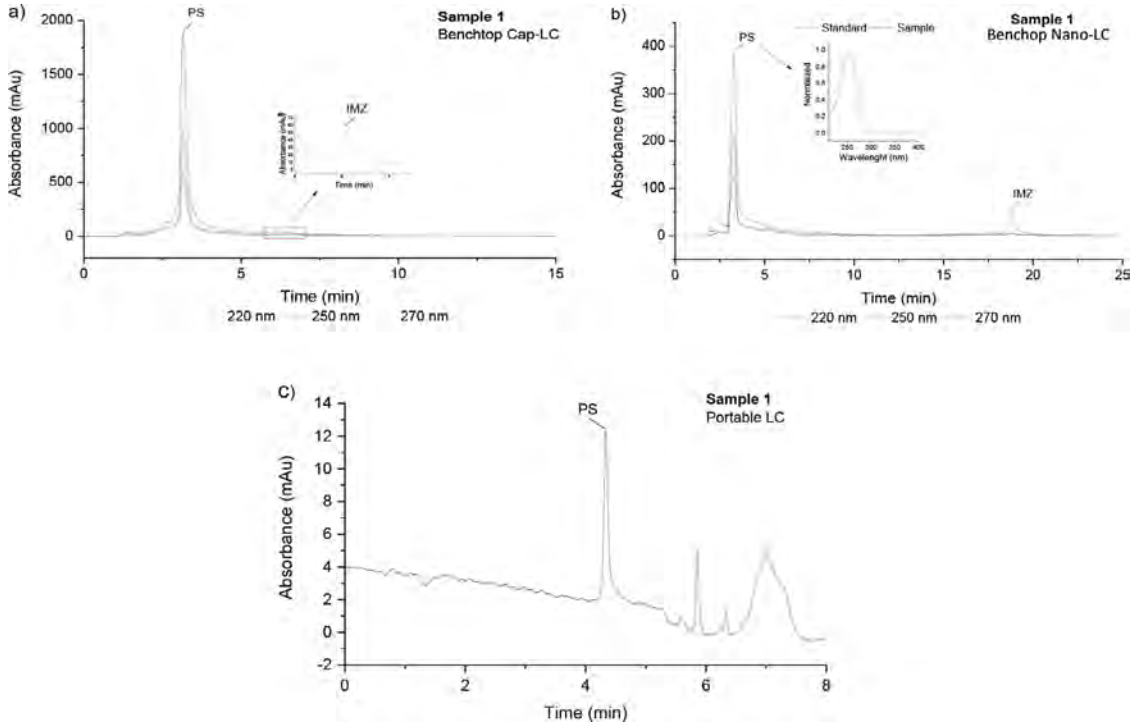

Fig. 4. Chromatograms obtained for "sample 1" using the benchtop NanoLC (150 × 0.1 mm, 3.5 µm column) and CapLC (35 × 0.5 mm, 5 µm column) and the portable LC. For more explanations see text.

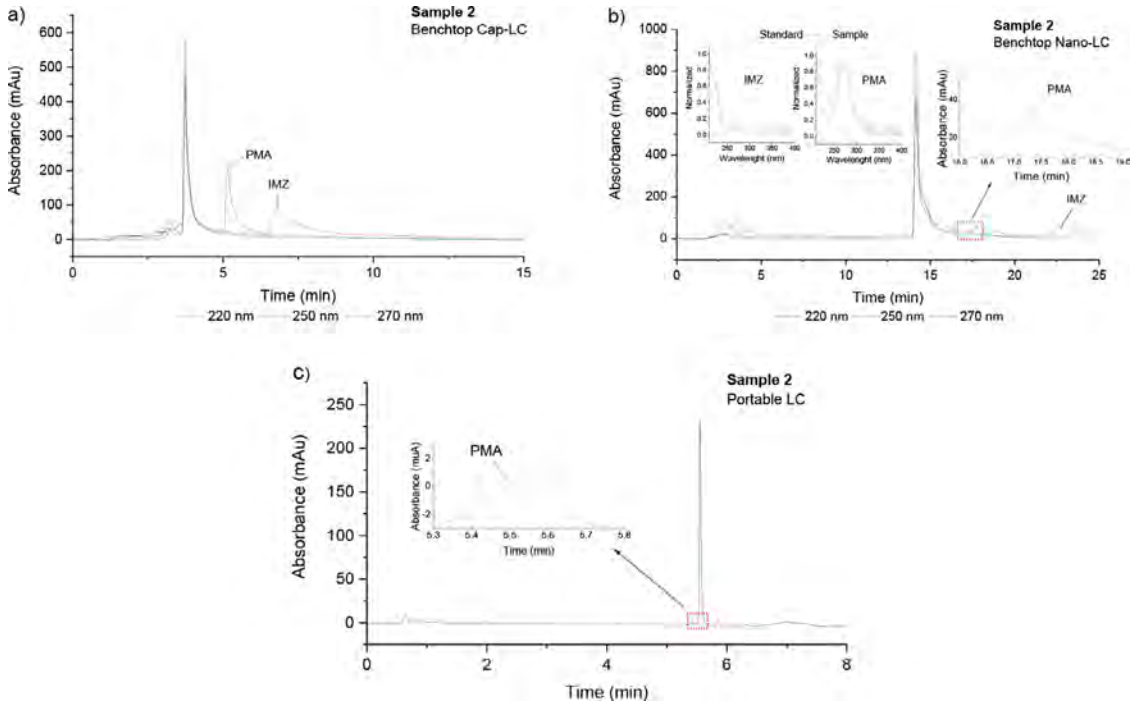

Fig. 5. Chromatograms obtained for "sample 2" using the benchtop NanoLC (150 × 0.1 mm, 3.5 µm column) and CapLC (35 × 0.5 mm, 5 µm column) and the portable LC. For more explanations see text.

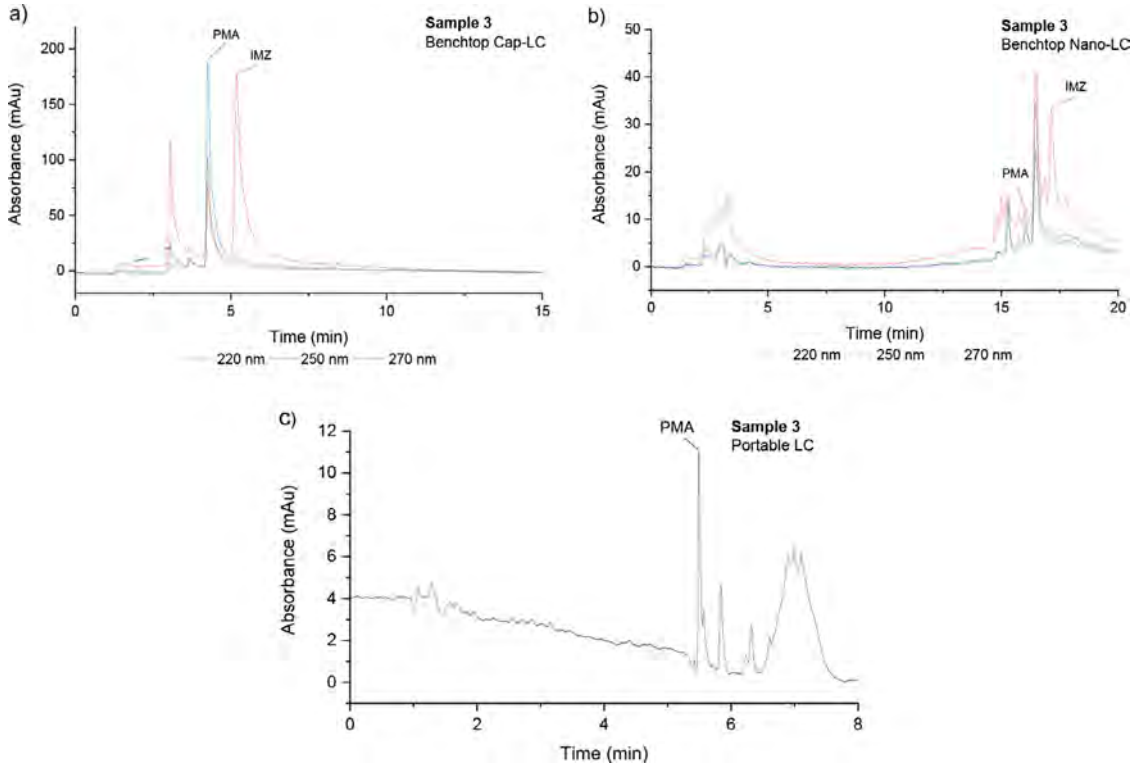

Fig. 6. Chromatograms obtained for "sample 3" using the benchtop NanoLC (150 × 0.1 mm, 3.5 µm column) and CapLC (35 × 0.5 mm, 5 µm column) and the portable LC. For more explanations see text.

of electricity of 0.15/kWh the Axcend Focus LC will cost 0.02 €/h which is less than 0.01 €/run. Because of the low solvent usage and injection volume, waste production is minimal (50 µL per run). The system weight is 8 kg without the cartridge, which is slightly higher than the maximum weight to consider an instrument as portable which is 7 kg [28], nevertheless, it can be well hand transported. Regarding to the operation time, the system can work away from main power for an average of 12 h, which is similar to the battery duration of the latest portable chromatographs (see Table 1). The chromatograph autonomy is enough to last a full working day. The equipment is robust at the climate conditions tested.

Criteria	GRADE 1	2	3	4	5
System cost		■			
Cost / test					■
Weight			■		
Portability				■	
Operation time		■			
Robustness Testing				■	
Performance			■		
Sample introduction			■		
Waste production				■	

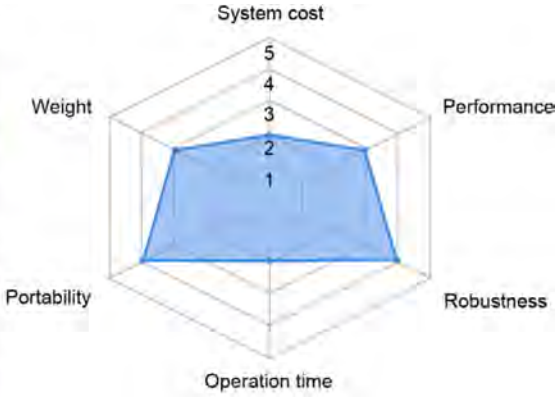

Fig. 7. Table and radar chart with the different assessments of BETTER criteria (higher grades means better results).

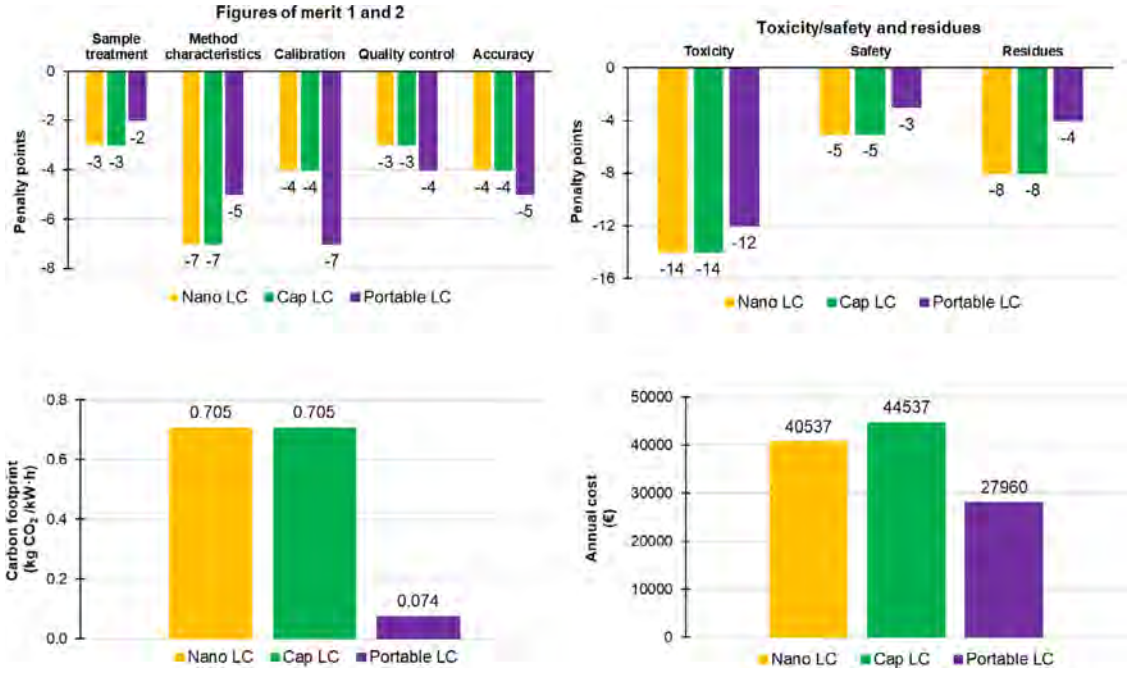

Fig. 8. Penalty points assignment to the variables evaluated through the hexagon tool for the three LCs.

Chromatographic performance is classified in accordance with the operation pressure. Portable LC can operate at 410 bar, which is the same value than those provided by the most of portable LC described [15]. The sample introduction consists of a closed injection system so the operator cannot modify anything. In general, the sample introduction is a simple procedure that does not require a highly specialized operator. Pretreatment should be included if the sample clean-up and/or preconcentration steps are needed. Fig. 7 sum up the different BETTER assessments in a table and a radar chart in accordance with [15]. BETTER grade levels (ranging from 1 to 5) describe the advancement of a given characteristic. Followed grade classification [15] can be found in https://better-hplc.github.io.

3.5.2. Hexagon pictogram

The HEXAGON tool was employed for evaluating and quantifying the associated features of an analytical methodology, in reference to its figures of merit, greenness and sustainability. Metrics are defined in Ballester-Caudet et al. [23], in which the better adaptation of all aspects for providing a reliable analytical result is found when the lower the penalization score [23,24]. An overall penalization ranking from 0 to 4 scale is indicated in a hexagon pictogram where variables such as figures of merit, health and safety, environmental impact, sustainability, and cost-benefit relation are shown. Eventually, the arithmetic mean (Sav) of the 0–4 scale is computed in order to compare different analytical methods [24].

Firstly, the penalty points (PPs) of the figures of merit 1 (FM-1) were assigned. We considered the problem to be solved: quantification of biocides at mg L^{-1} levels in washing waters. On the one hand, water sample processing involved filtering (0.45 µm pore-size nylon filter) and dilution with distilled water for all the analytical techniques studied. Thus, similar penalization score was obtained for the sample treatment evaluation within figures of merit 1, as represented in Fig. 8. Regarding calibration, portable minLC yielded penalization scores because of the higher limits of detection reached with respect to the other lab techniques.

Toxicity and safety evaluation were performed by collecting reagents pictograms and the assignment of the corresponding penalty points [23]. Generally, acetonitrile and methanol were employed as organic solvents, both implying health severe toxicity and flammable physical hazard. The amount of waste generated

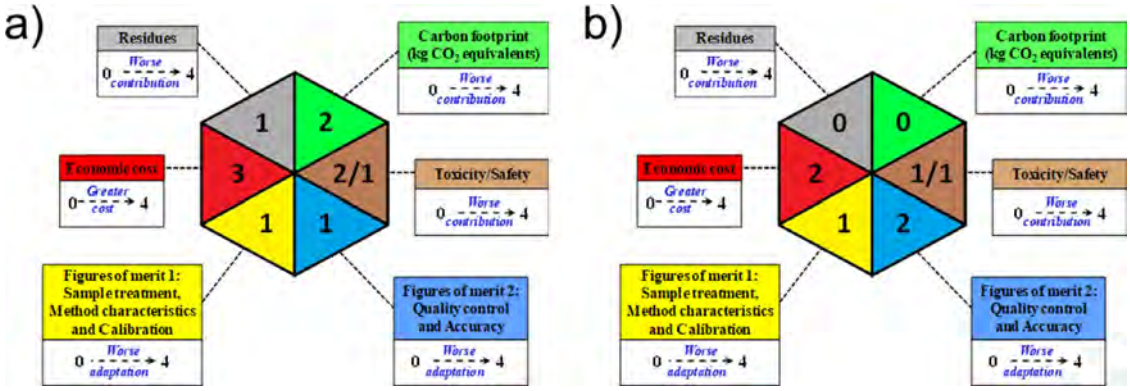

Fig. 9. Hexagon tool for capLC nanoLC (a) and Portable minLC (b).

was also considered and penalized within the residues variable of the HEXAGON tool. Solvent amount used and residue production were negligible for portable minLC in reference with benchtop LCs. This fact influences PPs for toxicity and safety estimations and residues as it is shown in Fig. 8. The environmental impact was also quantified by the so-called carbon footprint and expressed by kilograms of CO_2 equivalent. In this regard, electricity consumption is the main factor to be considered. The portable minLC showed the best adaptation to reduce environmental impact (Fig. 8). Finally, the cost-effectiveness of the analytical methodologies was analyzed according to the instrumentation needed, electricity consumption, salary of qualified personnel and reagents and materials costs. It was obtained that portable minLC was the cheapest choice (Fig. 8) due to the instrumentation is the cheapest and the electrical and the reagent consumptions and the analysis time are the lowest.

Final overall qualification in a 0–4 scale is indicated in the hexagon pictogram depicted in Fig. 9. The arithmetic mean is also calculated, which allows to rank the analytical methods according to greenness and sustainability aspects. Portable minLC (s_{av} = 1.0) is more green and sustainable than capLC and nanoLC (s_{av} = 1.57) for solving the planned problem.

4. Conclusions

In this work the capabilities of a portable minLC for in-field analysis were studied and the figures of merit were compared with the ones obtained for benchtop capLC and nanoLC. This study allowed to conclude that in comparison with the lab LCs, chromatograms obtained with the portable minLC had higher peak resolution, in a shorter analysis time with minimal solvent consumption, however its sensitivity is notably lower. Alternative LED's such as 235 and 275 nm, which are commercially available from Axcend, could improve signal-to-noise for certain analytes. All systems were suitable for the practical application selected. Regarding to the in-field analysis, portable minLC showed adequate performance at the several tested conditions. The results obtained from BETTER criteria for portability were suitable and the HEXAGON tool for testing greenness and sustainability provided better results for the portable minLC than those achieved by lab capLC and nanoLC for the planned problem: testing the biocide concentration of residual water from fruit treatments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

S. Cortés-Bautista: Methodology, Investigation, Validation, Writing – review & editing. **R. Navarro-Utiel:** Methodology, Investigation, Validation, Writing – review & editing. **A. Ballester-Caudet:** Methodology, Investigation, Validation, Writing – review & editing. **P. Campíns-Falcó:** Conceptualization, Supervision, Methodology, Investigation, Resources, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463119.

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