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Development and validation of a reversed-phase liquid chromatography method for the quantitative determination of carboxylic acids in industrial reaction mixtures

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Abstract

Usually analysis of low molecular-mass carboxylic acids was performed by anion-exchange or ion-exclusion chromatographic methods. Reversed-phase liquid chromatography (RPLC) was evaluated in this work as an alternative method for the analysis of low molecular-mass aliphatic mono- and di-carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, succinic, glutaric and adipic) in aqueous media. The separation of the nine organic acids was optimised in 21 min on a high-density C_{18} column with an elution gradient made up of $HClO_4$ aqueous solution 10^{-3} mol L^{-1} and acetonitrile. For the quantitation, external standard and standard addition methods were compared. Both methods gave similar results, so the most convenient method, external standard, was chosen for acids quantitation. Then the method had been validated and applied to the semi-quantitative analysis of formic and acetic acids and to the quantitative analysis of the others compounds in industrial reaction mixtures with concentrations ranging from 20 to 570 ppm.

Keywords: Reversed-phase liquid chromatography; Carboxylic acids; Quantitative analysis; Method validation

1. Introduction

Carboxylic acids belong to a class of compounds of biological and applicative importance. In the pharmaceutical industries, they were used as antioxidants, acidifiers and drugs. Carboxylic acids were also involved in industrial organic syntheses and carboxylates could be found as natural compounds, additives or preservatives in foods and beverages. Therefore numerous methods for the determination of carboxylic acids in environmental and biological samples had been developed. Carboxylic acids were usually separated by liquid chromatography methods: anion-exchange and ion-exclusion chromatography [1].

Ion-exclusion chromatography on acidic cation-exchangers was often recognised as a simple and convenient analytical technique for the analysis of carboxylic acids. Highly sulfonated styrene-divinyl benzene copolymer resins were classically used for the separation of these compounds. Retention of carboxylic acids on this type of stationary phase was based on both anion-exclusion chromatographic mechanism and hydrophobic interaction mechanism [2–4]. Unfortunately, the carboxylic acids with a long aliphatic chain were strongly retained on this type of resin and their peaks tailed strongly due to their hydrophobic nature [5]. The addition of organic solvent as alcohols [3,6,7] or acetonitrile [8–10] to the mobile phase is an easy and effective way for both improving the peak shape and reducing the retention. But in fact, only peak shape of the less hydrophobic acids was greatly improved. Moreover, the concentration of organic solvents in the mobile phase was limited because it caused the shrinkage of this type of stationary phase. In some cases, analysis of carboxylic acids could also be performed by adding to the mobile phase others compounds which strongly interact with the stationary phase and then

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reduce the apparent hydrophobicity of the resin [2,11,12]. But this approach was really very specific and too restricted in terms of application.

Anion-exchange chromatography with gradient elution [13,14] and conductivity detection in which a low conductivity eluent was used, had been proposed and applied to the determination of organic acids in various matrices as food samples [15], coffee or tea [16]. Basic pH of the mobile phase induced the dissociation of the acidic groups and therefore the carboxylic acids could be separated as anionic species according to their charge, their size and their polarisabilty. It had been shown that hydrophobicity and ion-exchange capacity of the stationary phase had a great influence on the separation of carboxylic acids, especially for divalent and unsaturated anions [17]. As for the ion-exclusion chromatography, the resin used and the composition of the mobile phase: eluting ion, pH, organic modifier, had a strong influence on the separation and on the resolution of carboxylic acids; but the effect of these parameters was not always well established [18].

The simultaneous separation of individual mono- and di-carboxylic acids was often difficult by anion-exchange or by ion-exclusion. Therefore, the aim of this work was to develop a simple and convenient method for the analysis of mono- and di-carboxylic acids in industrial reaction mixtures. These reaction mixtures contained many different compounds with a large polarity range, but only carboxylic acids were ionisable species. Indeed, the others compounds present in the industrial reaction mixture were essentially esters, with various aliphatic chains, more hydrophobic than carboxylic acids. Hydrophobicity of some carboxylic acids made difficult their separation by ion-exclusion or anion-exchange. So, use of this property as main retention mechanism could be interesting. Therefore a reversed-phase liquid chromatography (RPLC) with UV detection method was evaluated as an alternative to anion-exchange and ion-exclusion approaches, for the analysis of carboxylic acids in industrial reaction mixtures. This method offered some advantages as easy of use and faster analysis. Indeed, the compounds could be eluted with a simple water/acetonitrile gradient in suitable time with good peak shape. Moreover, the carboxylic acids, more hydrophilic than other compounds, could be eluted first. For method development, the separation optimisation was performed on C_{18} columns, and two quantitation methods (external standard and standard addition) were compared. After validation, the reversed-phase chromatography method was applied to the quantitation of the nine carboxylic acids in industrial reaction mixtures with concentrations ranging from 20 to 570 ppm.

2. Experimental

2.1. Chemicals

Formic, propionic, butyric, valeric, caproic, succinic and perchloric acids were purchased form Aldrich (Saint Quentin Fallavier, France), acetic acid was from Prolabo (Fontenay sous Bois, France) and glutaric and adipic acids were from Merck (Nogent sur Marne, France), with purity ≥99%. HPLC-grade solvent from Carlo Erba (Val de Reuil, France) and Milli-Q water, ultrapure water purification system (Millipore, Molsheim, France), were used for the mobile phase and to prepare the stock solutions of analytes.

2.2. Equipment and operating conditions

The LC system was composed of a HP 1050 quaternary pump, a HP 1050 autosampler and a HP 1100 variable-wavelength detector operated at $\lambda = 220$ nm with Chemstation 6.03 (Agilent Technologies, Waldbronn, Germany). The rate of data acquisition was at least 25 Hz. Concerning temperature regulation, the column was placed in a water jacket connected to a water bath Neslab (Courtaboeuf, France) RTE-101 set at $50 \pm 0.1\,^{\circ}C$. The flow rate was $1\,mL\,min^{-1}$. The separation was performed on a high-density C_{18} column, BetaMax Neutral (150 mm × 4.6 mm i.d., 5 μm) from Thermo-Electron Corporation (Courtaboeuf, France). A Symmetry Shield column (150 mm × 4.6 mm i.d., 5 μm) from Waters (Saint Quentin en Yvelines, France) was also evaluated. The mobile phases of the gradient were (A) $HClO_4 10^{-3}$ mol L^{-1} aqueous solution with 5% of acetonitrile and (B) acetonitrile with 5% of water. After the run the column was equilibrated under the starting conditions for 10 min. The injected volume was 10 μL.

To be analysed, industrial reaction mixtures sent by Rhodia, were diluted in the convenient solvent, corresponding to the mobile phase composition at the beginning of the elution gradient (H_2O/CH_3CN, 95:5 v/v), so that all the compounds were dissolved.

The statistical data analyses were performed on JMP 5.1 (S.A.S. Institute Inc., Carry, NC, USA) and on Excel 2002 (Microsoft Corporation, Courtaboeuf, France).

3. Results and discussion

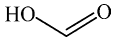
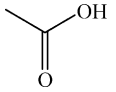
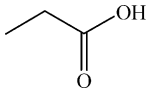
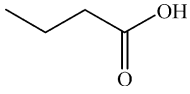
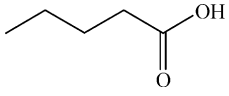
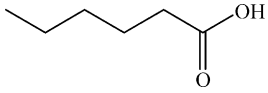
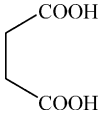
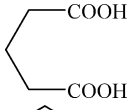
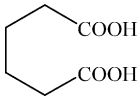
3.1. Method development

3.1.1. Optimisation of the separation

To develop a RPLC method for the simultaneous analysis of the C_1–C_6 mono-carboxylic and the C_4–C_6 di-carboxylic acids presented in Table 1, several parameters had to be considered. These compounds were low molecular-mass, poor chromophores, in each group of mono- or diacids the structure of the compounds just differed by a CH_2 group, their pK_a were close, and they covered a wide polarity range ($-0.59 < \log P < 1.92$ Table 1). The most polar compounds were usually poorly retained on classical C_{18} columns. So to have satisfactory retention on C_{18} column strong interactions had to be developed between the analytes and the stationary phase.

The addition of acid to the mobile phase lowered the pH and suppressed the ionization of the acidic functional groups

Table 1
Formula, pK_a and log P of analysed carboxylic acids

	Formula	pK_a	pK_a	log P
Formic		3.7		−0.54
Acetic		4.7		−0.17
Propionic		4.9		0.33
Butyric		4.8		0.79
Valeric		4.8		1.39
Caproic		4.9		1.92
Succinic		4.2	5.6	−0.59
Glutaric		4.3	5.4	−0.29
Adipic		4.4	5.4	0.08

of the solutes. Under the molecular form the retention and the separation of carboxylic acids was therefore based on their hydrophobicity. Indeed, the retention was the result of hydrophobic interactions between the hydrocarbonaceous moiety of the solute and the octadecyl chains of the stationary phase [19]. So, in order to have the carboxylic acids under the molecular form, the aqueous mobile phase contained perchloric acid (10^{-3} mol L^{-1}, pH 3).

Perchloric acid was chosen for its transparency at lower wavelength, because even if carboxylic acids were not good chromophores, UV detection at $\lambda = 220$ nm was a good compromise which allowed detecting all of them. Indeed, conductimetric detection could not be used because acids were not under ionic form. With mass spectroscopy only the diacids could, in our conditions, be detected (both with APCI and electrospray ionizations).

According to the wide polarity range of the solutes an elution gradient was necessary to elute all the compounds within a convenient time. The organic modifier chosen was acetonitrile because of its lower UV absorption than methanol at $\lambda = 220$ nm. But to control the retention of the most polar acids the elution gradient had to begin with a low rate of organic modifier.

To develop strong interactions between the solutes and the stationary phase two types of C_{18} columns had been selected: a Symmetry Shield (Waters) and a BetaMax Neutral (Thermo) columns [20]. The Symmetry Shield presented embedded carbamate groups that may increase the retention of polar compounds by polar interactions and tolerated pure water mobile phase. The BetaMax Neutral is a high-density C_{18} column which strongly retained compounds by hydrophobicity. Carboxylic acids separation was performed with both columns. For the BetaMax Neutral column elution gradient began with $HClO_4$ 10^{-3} mol L^{-1} aqueous solution with 5% acetonitrile, whereas for the Symmetry Shield column the elution gradient began with only $HClO_4$ 10^{-3} mol L^{-1} aqueous solution. The chromatograms obtained under the same conditions of temperature and flow rate have been presented on Fig. 1. For both columns elution order was the same, monocarboxylic acids on the one hand and di-carboxylic acids on the other hand were eluted according to their polarity (log P, see Table 1), the most polar compounds eluted first. In spite of the presence of carbamate groups and the use of a mobile phase which contained pure water the separation of carboxylic acids was less satisfactory on the Symmetry Shield column than on the BetaMax Neutral column: formic acid was not detected, and propionic and glutaric acid were coeluted.

Consequently the column chosen for the analysis of the carboxylic acids was the Betamax Neutral, and the separation conditions adopted were gathered in Table 2.

3.2. Choice of the quantification method

3.2.1. Linearity of the response function

A standard mixture of the nine carboxylic acids was diluted to give five samples at five different concentrations, for each concentration three replicates were carried out. The linearity of the response curves was evaluated by plotting the peak area corresponding for each analyte, as a function of the its introduced concentration. The data were analysed by linear regression. The slope, the intercept, and the coefficient of determination were calculated. Coefficient of determination were above 0.999 except for butyric acid ($R^2 = 0.998$)

Table 2
Operating conditions for the RPLC method

	Elution gradient	
	Solvent A: $HClO_4$ 10^{-3} mol L^{-1}/5% CH_3CN	Solvent B: CH_3CN/5% water
0 min	100	0
5 min	100	0
10 min	90	10
15 min	75	25
20 min	60	40
25 min	40	60
30 min	20	80
35 min	10	90
40 min	10	90
Equilibration time 10 min	100	0

Column: BetaMax Neutral (150 mm × 4.6 mm i.d., 5 μm); flow rate: 1 mL min^{-1}; temperature: 50 °C.

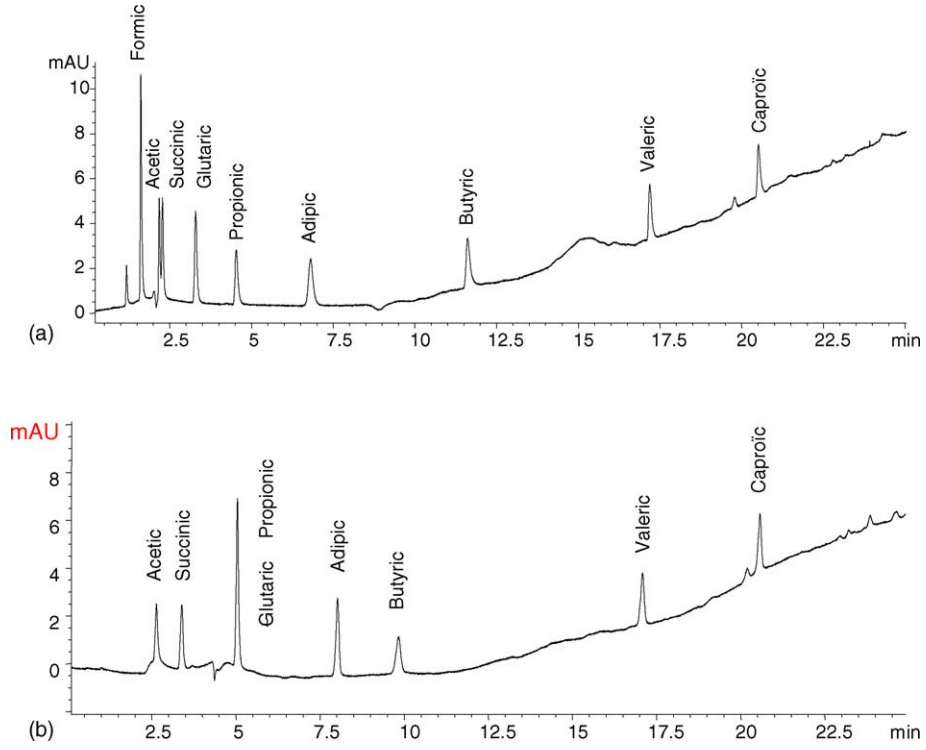


Fig. 1. Separation of nine carboxylic acids on (a) C_{18} BetaMax Neutral column (150 mm × 4.6 mm i.d., 5 μm) and (b) C_{18} Symmetry Shield column (150 mm × 4.6 mm, i.d., 5 μm). Mobile phase (A) $HClO_4$ aqueous solution 10^{-3} mol L^{-1} + 5% CH_3CN; (B) CH_3CN + 5% water, flow rate = 1 mL min^{-1}, $T = 50\,^{\circ}C$.

and the lack of fit test [21,22] was not significant (Prob > F above 0.01) except for formic and acetic acid (Prob > F below 0.01). So there was a linear correlation between the peak area and the carboxylic acid concentration. Student's test showed that the intercept was not significantly different from 0 except for formic and acetic acids, peaks of which were affected by injection perturbations.

3.2.2. Choice of the quantitation method

In order to choose the best way to quantify carboxylic acids in industrial reaction mixtures, two methods were compared: external standard quantitation [23] and quantitation by standard addition [24]. Then two calibration curves were carried out by plotting peak area as a function of carboxylic acid concentration. For the first one, the standard solution of the nine carboxylic acids was diluted at different concentrations in Milli-Q water. For the second one, known amounts of the standard solution were added, in the same range of concentrations, to the actual industrial reaction mixture. This procedure made possible the comparison of calibration curves in pure water media (external standard quantitation) and in real sample media (quantitation by the standard addition method) where other compounds were present. It was then possible to control if other compounds interfered with the target analytes and modified the quantification.

To compare the two methods a statistical approach was developed. Statistical tests were carried out at the 95% confidence level (corresponding to a first kind risk $\alpha = 5\%$). On the first step the variances σ_1^2 (standard addition method) and σ_2^2 (external standard) of the two slopes were compared thanks to a F-test. The observed value $F_{obs} = \sigma_1^2/\sigma_2^2$ was compared to the $F_{critical}$ value of the Snedecor variable with (ν_1, ν_2) degrees of freedom. If $F_{obs} < F_{critical}$ the F-test did not show significant difference between the two variances and the pooled variance σ could be calculated (Eq. (1)).

$$\sigma = \left(\frac{\nu_1 \sigma_1^2 \nu_2 \sigma_2^2}{\nu_1 + \nu_2} \right)^{1/2} \quad (1)$$

If the variances were compatible, it was then possible to go to the second step. Slopes s_1 (standard addition) and s_2 (external standard) of the two calibration curves were compared thanks to a t-test. If the Student's function t_{obs} (Eq. (2)) is below the $t_{critical}$ value at ($\nu_1 + \nu_2$) degrees of freedom, the two slopes could not be considered as different.

$$t_{obs} = \frac{s_1 - s_2}{\sigma\sqrt{2}} \quad (2)$$

Finally if t-test did not show significant difference between the two slopes, the quantification of carboxylic acids in the industrial reaction mixture was performed both by external standard and by standard addition and the results were compared. If the t-test showed significant difference between s_1 and s_2, this difference was evaluated. Then, if it was less than 5% the quantitation by both methods could

be carefully carried out and the difference between obtained results could be evaluated.

For example, calibration curves obtained by external standard ($y = 0.2158x - 0.0117$, $R^2 = 0.9991$) and by standard addition ($y = 0.2233x + 15.527$, $R^2 = 0.9975$) were carried out for succinic acid. Statistical tests were performed at the 95% confidence level (corresponding to a first kind risk $\alpha = 5\%$). Variances and slopes estimates and statistical test results were gathered in Table 3. F_{obs} was inferior to $F_{critical}(3, 4, 0.05)$, so the variances σ_1^2 and σ_2^2 could not be considered as different, σ could be calculated and slopes s_1 and s_2 could be compared. t_{obs} was also inferior to $t_{critical}(7, 0.05)$, slopes s_1 and s_2 could not be considered as different; so calibration curves were compatible. Quantitation of succinic acid in an industrial reaction mixture was performed both by standard addition and by external standard. The obtained results were equivalent (Table 3). So the quantification of succinic acid in industrial reaction mixtures could be performed by external standard method which was more convenient.

The same procedure applied to other carboxylic acids, did not show differences between external standard quantitation and quantitation by the standard addition method for all the carboxylic acids, except for formic and acetic acids for which t_{obs} was superior to $t_{critical}$. The difference observed between the two slopes was evaluated at 3.6% for formic acid and at 4.3% for acetic acid. Then quantitations of formic and acetic acid were performed by both methods and the observed differences between results were respectively evaluated at 17% and 11%.

Therefore, quantitation by external standard was a convenient method to analyse carboxylic acids in industrial reaction mixtures which gave satisfactory results for seven carboxylic acids. Formic and acetic acids were poorly retained, their peaks were affected by injection perturbations which were more intensified for the industrial reaction mixtures injection. So, only a semi-quantitative analysis could be performed for formic and acetic acids.

Table 3
Statistical approach to compare quantitation by standard addition method and by external standard method

	σ	$F_{critical}(3, 4, 0.05) = 6.59$	Significant
Standard addition	$\sigma_1 = 0.0035$	$F_{obs} = 1.76$	No
External standard	$\sigma_2 = 0.0026$		
	$\sigma = 0.0030$		
	s	$t_{critical}(7, 0.05) = 2.36$	Significant
Standard addition	$s_1 = 0.2158$	t_{obs}=1.78	No
External standard	$s_2 = 0.2233$		
	Quantitation (ppm)		
Standard addition	69 ± 2		
External standard	72 ± 1		

Uncertainties given on quantitation values were based on 95% confidence intervals.

3.3. Method validation

The second aim of this work was to validate the developed method in order to apply it to the quantitation of the carboxylic acids in industrial reaction mixtures. Depending on the compounds, concentration could vary from 20 to 570 ppm. Precision and accuracy of the method had to be better than 5%.

3.3.1. Selectivity

For the nine compounds, the minimum resolution was observed between the peaks of acetic and succinic acids, and the value was never less than 1.2, which was acceptable for UV detection at $\lambda = 220$ nm. Lowering the pH at 2.5 did not improve this resolution.

3.3.2. Precision

The precision of the method was evaluated for a concentration of about 50 ppm by making repeated analyses on different days. Standard mixtures of the nine carboxylic acids were prepared each day, analysed through five replicates ($n = 5$) during three days ($n_1 = 3$) and used to calculate the day-to-day repeatability and the intermediate precision. The responses measured on each chromatogram were the retention time of each peak and the corresponding area under each peak. An analysis of variance (ANOVA) [25,26] was made and the results were used to calculate the different parameters of the precision. The intra-day (Eq. (3)) and the day-to-day (Eq. (4)) dispersions expressed as relative standard deviations were respectively evaluated from the residual error (σ_r) and from the error due to the day factor (σ_A), and then the intermediate precision (Eq. (5)) was calculated as follows.

$$\mathrm{RSD}_{\text{intra-day}}(\%) = \frac{\sigma_r}{\bar{x}} \times 100 \quad (3)$$

$$\mathrm{RSD}_{\text{day-to-day}}(\%) = \frac{\sigma_A}{\bar{x}} \times 100 \quad \text{with} \quad \sigma_A^2 = \frac{q_A - q_r}{n} \quad (4)$$

$$\mathrm{RSD}_{\text{intermediate precision}}(\%) = \frac{\sqrt{\sigma_A^2 + \sigma_r^2}}{\bar{x}} \times 100 \quad (5)$$

$\bar{x}$: mean of response; q_A: day factor mean square given by ANOVA; q_r: residual mean square given by ANOVA.

Results for t_r and area precisions were summarised in Table 4. Very little dispersion was observed for retention times because intra-day, day-to-day dispersions and intermediate precision were less than 1% (except for butyric $\mathrm{RSD}_{\text{intermediate precision}}$ was 1.05%) which could allow an easy identification of the compounds. For peak areas, intra-day and day-to-day dispersions were in the same range inferior to 3%, intermediate precision ranged from 1 to 3.5% depending on the compounds.

Performances of the method were sufficient for the analysis of carboxylic acids because intermediate precision of the method was always less than 5%.

Table 4
Results of the analysis of intra-day ($n = 5$) and day-to-day ($n_1 = 3$) dispersions and intermediate precision

Carboxylic acid	RSD (%) intra-day (t_r)	RSD (%) day-to-day (t_r)	RSD (%) intermediate precision (t_r)	RSD (%) intra-day area	RSD (%) day-to-day area	RSD (%) intermediate precision area
Formic	0.34	0.56	0.65	0.82	1.04	1.33
Acetic	0.33	0.48	0.58	1.20	NS	1.20
Succinic	0.33	0.36	0.49	1.67	NS	1.67
Glutaric	0.35	NS	0.35	0.63	0.93	1.12
Propionic	0.33	0.31	0.45	1.58	1.34	2.07
Adipic	0.29	NS	0.29	0.75	1.65	1.81
Butyric	0.63	0.84	1.05	2.54	NS	2.54
Valeric	0.57	NS	0.57	2.02	2.72	3.38
Caproic	0.47	NS	0.47	2.11	2.58	3.34

NS: non significant.

3.3.3. Linearity

In this case, the method was applied to the analysis of carboxylic acids in industrial reaction mixtures and it had to be able to quantify these compounds from 20 to 570 ppm. Analytical ranges were given for each carboxylic acid in Table 5. The linearity of the method was evaluated in these concentration ranges by plotting concentrations obtained by applying the whole method against introduced concentration for each carboxylic acid. For each concentration three independent solutions were prepared. We expected a linear response with a slope of 1 and a 0 intercept, if the method was linear and accurate. Then linear regressions were performed, results on slope and intercept intervals and on the coefficient of determination were given in Table 5. Good coefficients of determination were obtained greater than 0.996 (butyric and adipic acids), even greater than 0.999 (other acids). The lack of fit test was not significant except for formic acid (see Table 5). For all the carboxylic acids slope was not significantly different from 1 and intercept was not significantly different from 0. So the linearity of the method was established for all the carboxylic acids in the range needed for their quantitation in industrial reaction mixtures.

Here it was not looked for the smallest quantity of solute that could be quantified. However, it was checked that at the lower limit of the analytical range, defined as method limit of quantification (LOQ) [27], analytical performances were satisfactory. Consequently, for the first concentration of the analytical range, the signal to noise ratio (S/N) was measured and the relative standard deviation of the peak area was calculated with five replicates. Results obtained for each carboxylic acid at the lower concentration were presented in Table 6. Signal to noise ratio ranged from 17 to 155 and repeatability RSD from 0.4% to 3.5%. The signal to noise ratio was always more than 10 and the RSD less than 5%. Consequently the inferior limits of the analytical ranges studied fulfilled the required criterion for the method, and the quantitation at these concentrations could be carried out in a satisfactory way.

3.3.4. Accuracy

To evaluate the accuracy of the method, a synthetic industrial reaction mixture free from the nine carboxylic acids was prepared. This means, that the compounds present in real industrial reaction mixtures, except the carboxylic acids,

Table 6
Signal to noise ratio and RSD value for the lower concentration of linear range

Carboxylic acid	Concentration (ppm)	S/N	RSD (%)
Formic	35	70	0.52
Acetic	20	17	2.8
Succinic	25	38	1.9
Glutaric	115	155	0.4
Propionic	35	33	0.86
Adipic	110	83	1.2
Butyric	35	25	1.25
Valeric	35	35	3.50
Caproic	40	47	1.25

Table 5
Results of linearity ranges and linear regression

Carboxylic acid	Range (ppm)	Slope confidence interval	Intercept confidence interval	R^2	Lack of fit	
					Prob > F	Significant
Formic	35–200	[0.998; 1.021]	[−2.01; 0.81]	0.999	0.03	Yes
Acetic	20–90	[0.989; 1.027]	[−1.18; 1.31]	0.999	0.26	No
Succinic	25–210	[0.992; 1.017]	[−2.47; 0.87]	0.999	0.35	No
Glutaric	115–570	[0.996; 1.024]	[−7.56; 2.70]	0.999	0.31	No
Propionic	35–280	[0.984; 1.017]	[−2.64; 2.26]	0.999	0.08	No
Adipic	110–540	[0.992; 1.050]	[−12.58; 8.36]	0.998	0.07	No
Butyric	35–280	[0.981; 1.014]	[−2.67; 2.24]	0.996	0.16	No
Valeric	35–290	[0.984; 1.017]	[−3.06; 1.89]	0.999	0.87	No
Caproic	40–310	[0.997; 1.027]	[−3.52; 1.39]	0.999	0.08	No

Table 7
Accuracy results: spiked and measured concentrations of carboxylic acids in a synthetic industrial reaction mixture prepared free from these carboxylic acids

Carboxylic acid	Spiked concentration (ppm)	Measured concentration (ppm)	Recovery (%)	Spiked concentration (ppm)	Measured concentration (ppm)	Recovery (%)
Formic	195	205	105	90	93	103
Acetic	40	58	145	20	28	140
Succinic	130	131	101	60	58	97
Glutaric	535	533	99	240	239	100
Propionic	75	73	97	245	243	99
Adipic	305	303	99	140	143	102
Butyric	110	111	101	205	207	101
Valeric	90	90	100	180	184	102
Caproic	100	102	102	195	197	101

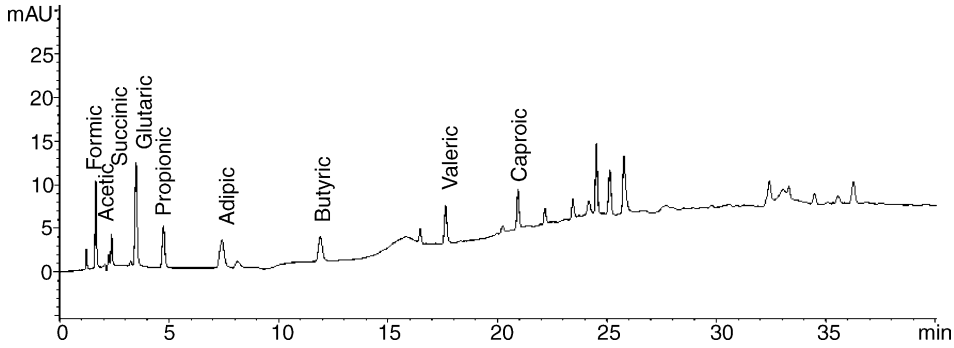


Fig. 2. Chromatogram of an industrial reaction mixture which contained carboxylic acids and others compounds. C_{18} BetaMax Neutral column (150 mm × 4.6 mm i.d., 5 μm). Mobile phase (A) $HClO_4$ aqueous solution 10^{-3} mol L^{-1} + 5% CH_3CN; (B) CH_3CN + 5% water, flow rate = 1 mL min^{-1}, T = 50 °C.

were dissolved in the convenient solvent (H_2O/CH_3CN, 95:5, v/v). This sample was then spiked with the acids at two different concentrations (one in the lower part of the analytical range, the other in the upper part). Concentrations of carboxylic acids in the synthetic mixture had been determined using the calibration curves obtained with the external standard method. Results comparing introduced concentrations and measured concentrations were gathered in Table 7. Results were in good agreement (except for acetic acid) because recovery was between 97 and 105%, so the error was less than 5%. Only acetic acid quantitation was not really satisfactory (recovery 140%). This result was not surprising because as previously shown, the analysis of acetic acid could be only semi-quantitative.

The reversed-phase liquid chromatography method was now validated, which ensured its performance level was compatible with its objectives.

3.4. Application to an industrial reaction mixture

Consequently, the method was applied to the analysis of the carboxylic acids in an industrial reaction mixture. Industrial sample was essentially made up of water and contained others compounds than the carboxylic acids. The chromatogram of an industrial reaction mixture was presented Fig. 2. Concentrations determined by the external standard quantitation method were gathered in Table 8.

Table 8
Application: quantitation of carboxylic acids in an industrial reaction mixture

Carboxylic acid	Measured concentration (ppm)
Formic	134
Acetic	36
Succinic	85
Glutaric	335
Propionic	203
Adipic	202
Butyric	150
Valeric	148
Caproic	163

4. Conclusion

An alternated reversed-phase liquid chromatography method had been developed and optimised to analyse nine carboxylic acids in industrial reaction mixtures. The separation was achieved in 21 min. It had been demonstrated that quantitation by external standard and the standard addition method gave identical or quite close (for formic and acetic acids) results. Consequently the external standard method, more convenient, could be used for the semi-quantitative analysis of formic and acetic acids, and for the accurate quantitation of the others acids in industrial samples. The analytical method was validated by demonstrating selectivity, precision, linearity and accuracy, which ensured its performances were compatible with its objectives in

the working concentration range. This method, fast and accurate, appeared to be a good alternative to other analytical methods as anion-exchange or ion-exclusion.

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