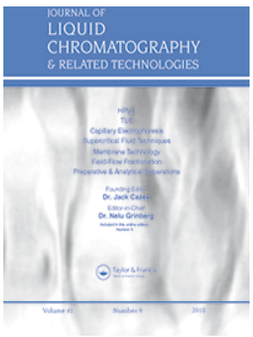

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Determination of formic, acetic, propionic, and *n*-butyric acids by indirect photometric chromatography

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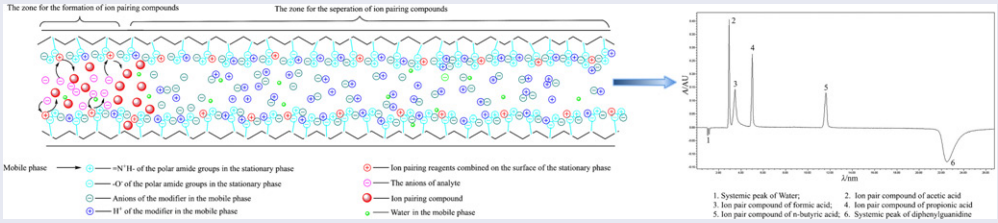
ABSTRACT

An indirect photometric chromatography (IPC) method was developed for the determination of formic, acetic, propionic and *n*-butyric acids using high-performance liquid chromatography with ultraviolet detection. The chromatographic separations were performed on a Waters SymmetryShield PR_8 column with a diphenylguanidine aqueous solution as the mobile phase and the pH value was adjusted by *n*-butyric or *n*-pentanoic acid. The forming mechanism of the ion-pairing compounds was discussed. The effect of the column, the kind, and concentration of ion-pairing reagents and modifiers were investigated. The results showed that the linear relationships of the four kinds of acids all performed well and the correlation coefficients (R^2) were all above 0.996. The established method had a good precision and accuracy for the acids. The IPC method was also compared with a titrimetric method for determining the sample content. Additionally, to eliminate the errors caused by the different backgrounds between the standard solutions and complex sample solutions, a standard additive method was explored for the analysis of a mixture of acids. The established method is simple, efficient, and accurate for determining C_1 to C_4 linear chain saturated monocarboxylic acids.



KEYWORDS

Saturated monocarboxylic acids; diphenylguanidine; indirect photometric chromatography; HPLC-DAD

Introduction

Formic, acetic, propionic, and *n*-butyric acids are important fundamental organic chemical products used in the pesticide, medicine, food, leather, rubber, printing and dyeing, coating material industries, etc. The purity of the industrial raw materials decides the sale price and affects the quality of the deep processing products. It is necessary to establish an effective and accurate method for determination of these kinds of acids.

Many studies have reported methods for the determination of organic acids in different matrixes, such as the fermentation of sugarcane juice,[1] Italian beers,[2] yuzu juices,[3] anaerobe medium,[4] and soy sauce,[5] which were separated by high-performance liquid chromatography (HPLC) and monitored at a 210 nm wavelength by ultraviolet detection (UV). The organic acids of high degree of dissociation can be analyzed by ion exclusion chromatography or ion exchange chromatography[6,7]. Organic acids can also be determined by capillary electrophoresis, gas chromatography-mass spectrometry, titrimetric methods, etc.[6,8–11] The titrimetric method is suitable for the analysis of macro-organic acids, but the content measured by this technique is the total acid concentration. The above-mentioned methods are all used to analyze the compounds themselves directly. In addition, there is an indirect method for the determination of organic acids in seawater by precolumn derivatization, and then, the products were detected by HPLC-UV[12]. The derivative method avoids quantitatively analyzing organic acids at the low end of ultraviolet spectrum. Among the reported methods, few studies reported the indirect photometric chromatography (IPC) method for the analysis of organic acid by HPLC.

The concept of IPC was proposed by Small in 1982,[13] and at the beginning, it was used to determine inorganic ions with an ion-exchange column and UV detection. This method was realized by creating ion-exchange sites on the surface of the stationary phase of column.[14–18] Since the early 1990s, this method was applied to the analysis of organic ions and non-ionic compounds without ultraviolet absorption by the liquid distribution mode.[19–23] The generally recognized separation

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mechanisms are the dynamic ion-exchange mechanism and the ion-pairing formation mechanism.[24] Although the IPC method was proposed over 30 years ago, it has not been used extensively. The main reasons that limited the development of IPC were the lack of universal ion-pairing reagents and appropriate stationary phases. The ion-pairing reagent is related to the properties of analytes and should be easily substituted from the column to react with the analytes to form ion-pairing compounds. Different ion-pairing reagents can be simply applied to specific analytes.[19–23,25–27] In addition, the stationary phase of the column needs to tolerate washing with aqueous solutions containing a few ion-pairing reagents and modifiers for an extended time and needs to possess weak electric charges. With the improvement in bonding technology, the C_8 or C_{18} linear chain alkanes embedded with polar amide groups can be bonded onto the surface of ultrapure silica gel. This kind of column has a wide application range of pH values and more electric charges on the surface of the stationary phase.[28,29] This kind of column was suitable for this experiment.

To measure the contents of formic, acetic, propionic, and *n*-butyric acids efficiently and accurately, we established an IPC method based on the newly explored ion-pairing reagents and non-polar chromatographic columns embedded with polar amide groups. The forming mechanism of the ion-pairing compounds and influencing factors of the method were discussed. The optimum conditions of chromatography were also verified. The developed method realized the goal for determination of the nonconjugated organic anionic compounds by ultraviolet detection.

Experimental

Equipment

All experiments were carried out on a Waters 600 HPLC system (Waters, MO), which consisted of a quaternary HPLC pump, a 7725i six-way valve inlet (Rheodyne, WA) and a 2996 diode array detection (DAD) (Waters, MO). Ultrapure water was prepared by a Flom ultra-water apparatus (Flom Co., Qingdao, China) and filtered with 0.25-μm nylon filter.

Reagents

Formic acid (≥98.0%), acetic acid (≥99.8%), propionic acid (≥99.5%), *n*-pentanoic acid (≥99.0%) and diphenylguanidine (≥99.0%) were purchased from Sinopharm (Shanghai, China). *N*-butyric acid (≥99.0%) was purchased from Basf (Tianjin, China). Sodium hydroxide (≥98.0%) was acquired from Piet (Shanghai, China). Potassium hydrogen phthalate (≥99.5%) as a working standard reagent was purchased from Sanpu (Shanghai, China). Acetonitrile (HPLC grade) was obtained from Tianjin Four Fine (Tianjin, China). The samples were supplied by a chemical plant.

Chromatographic conditions

The separation conducted on the column of a Waters SymmetryShield RP_8 (4.6 mm ×150 mm, 3.5 μm particle size, Waters, MO). The mobile phases for the analysis of formic, acetic, propionic, and *n*-butyric acids were aqueous solutions of 0.12 mmol/L diphenylguanidine modified with 0.5, 1.0, 1.0 mmol/L *n*-butyric acid, and 1.0 mmol/L *n*-pentanoic acid. The mobile phase was degassed by Helium bubbling. The injection volume was 10 μL. The diode array detection (DAD) was set to scan in the wavelength range of 190–400 nm and the chromatogram was extracted from 232 nm. The resolution of the detection was 1.2 nm. The data acquisition and peak integration were performed with the help of Millenium 32® software (Chinese version, Waters, MO).

Preparation of the mobile phase

Because it was difficult to dissolve diphenylguanidine in ultrapure water, high-concentration solution was prepared first and then diluted for use. The diphenylguanidine solution with a concentration of 0.48 mmol/L was prepared by accurately weighing 0.25 g diphenylguanidine into 2.5 L ultrapure water. Diphenylguanidine was completely dissolved by ultrasonic vibration for 20 min, and the solution rested overnight. The high-concentration solution was diluted fourfold with ultrapure water to prepare the mobile phase. The pH of the mobile phase was adjusted with acids of the appropriate kind and concentration.

Preparation of the standard and sample solutions of each analyte

Stock solutions of each acid were prepared at a concentration of 2 mg/mL independently by dissolving appropriate amounts of analytes in ultrapure water. The stock solution of formic acid was diluted 20-fold with ultrapure water to obtain the standard solution, and the other acids were diluted tenfold. The steps for the preparation and dilution of sample solutions were the same as that of the corresponding standard solutions.

Preparation of the titrant and sample solutions for the titration method

A saturated sodium hydroxide solution was prepared by weighing 110 g sodium hydroxide into 100 mL carbon dioxide-free water. The clear supernatant (54 mL) was pipetted into a 1000-mL volumetric flask and diluted with carbon dioxide-free water to the mark. The titrant with a concentration of 1.0 mol/L was prepared for the determination of the sample content of formic acid, which was calibrated with a reference reagent, – potassium acid phthalate. The similar step was adopted to prepare a titrant with a concentration of 0.5 mol/L for the sample of acetic and propionic acid.

The sample solution of each acid was prepared by accurately weighing 1.0 g of the analyte and then dissolving the analyte in 80 mL carbon dioxide-free water. The preparation of each sample was repeated three times.

Preparation of the mixed solutions for the standard additive method

The mixed standard solutions of propionic acid were prepared for the establishment of the standard curve for the standard additive method in the concentration range of 50–1000 mg/L, and each solution was fortified with formic, acetic, and *n*-butyric acids at concentrations of 20, 200, 200 mg/L, respectively. The preparation method of the mixed standard solutions of *n*-butyric acid was the same as that of propionic acid, and the formic, acetic, and propionic acids were added to each solution as the background solutions. To validate the accuracy of the standard additive method, five mixed solutions were prepared with the four kinds of acids of different concentrations. The contents of propionic and *n*-butyric acid in the mixtures were quantified with the two standard curves prepared above.

Results and discussion

Fundamental principle of IPC

IPC is a special kind of ion pair chromatography. The ion-pairing reagents need to possess conjugate groups and absorb ultraviolet light. According to the fundamental principle of ion-pair chromatography, weakly acidic analytes can only react with a strongly basic ion-pairing reagent to form an ion-pairing compound. The selected ion-pairing reagent – diphenylguanidine – is a strong organic base and the pK_a value is 10.12. Diphenylguanidine has two phenyl groups and the maximum wavelength of ultraviolet absorption is at 232 nm.[30] Protonated diphenylguanidine and the modifier anions in the mobile phase combined with the amide groups on the surface of the stationary phase shape the electric double layer. When the solution was injected into the system, the analytes reacted with protonated diphenylguanidine on the head of Waters Symmetryshield RP_8 column yielding ion-pairing compounds and were separated after the head of the column. The formed compounds were monitored by DAD.

Optimization of the chromatographic conditions

The analytical condition of formic, acetic, propionic and *n*-butyric acids was optimized from the aspects of the chromatographic columns, ion-pairing reagents and modifiers. Meanwhile, the change rule of the response signal of ion-pairing compounds was investigated.

Selection of the chromatographic columns

During the optimization process, propionic acid was used as an example. Seven different chromatographic columns were applied under the same analytical conditions, that is, Waters SymmetryShield RP_8 (Symmetry RP_8), Waters Xselect CSH C_{18} (4.6 mm ×75 mm, 2.5 µm particle size, Waters, MO) (Xselect C_{18}), Waters XBridgeShield RP_{18} (4.6 mm ×150 mm, 3.5 µm particle size, Waters, MO) (XBridge RP_{18}), Waters XTerra RP_{18} (3.9 mm ×150 mm, 5 µm particle size, Waters, MO) (XTerra RP_{18}), Thermo Hypersil GOLD aQC_{18} (4.6 mm ×250 mm, 5 µm particle size, Thermo, MA) (Hypersil C_{18}), Elite SinoChrom ODS-BP C_{18} (4.6 mm ×250 mm, 5 µm particle size, Elite, Dalian, China) (SinoChrom C_{18}), and Agela Venusil MP C_{18} (4.6 mm ×250 mm, 5 µm particle size, Agela, Tianjin, China) (Venusil C_{18}). By comparing the chromatograms, it was found that the separation capacity of Xbridge RP_{18} and Xselect C_{18} column was similar to that of Symmetry RP_8. The retention time of propionic acid of the three columns was close to each other. The Xbridge RP_{18} and Xselect C_{18} columns can be used to verify the results acquired with symmetry RP_8. The retention time of propionic acid separated with XTerra RP_{18} was too short, that is, only 2.5 min. The response signal of the analyte was easily interfered with by impurities with short retention times. The retention time of propionic acid acquired with Venusil C_{18} was too long to reduce the work efficiency. Although the SinoChrom C_{18} column can be used in this experiment to obtain a chromatogram of the ion-pairing compounds, this kind of column could not tolerate a mobile phase of a 100% aqueous solution. By a comprehensive analysis, the Waters SymmetryShield RP_8 was selected as the analytical column in this experiment.

Optimization of ion-pairing reagents

The ion-pairing reagent was screened from many basic compounds with ultraviolet absorption, such as benzyltrimethylammonium bromide, tyramine hydrochloride, brilliant green, sulfaguanidine, *p*-anisidine, and diphenylguanidine. When adding the ion-pairing reagents, except diphenylguanidine, into the mobile phase, the organic acids directly flowed out of the column without retaining, and the peaks were detected with a low response value at an ultraviolet absorption wavelength of 210 nm. Therefore, was judged that the ion-pairing reagents did not react with the organic acids. Diphenylguanidine was the only compound that can combine with short-chain carbonic acids forming ion-pairing compounds on the column of the Waters SymmetryShield RP_8. This analytical method prolonged the retention time of the organic acids and shifted the ultraviolet absorption to longer wavelengths. The further step was to optimize the concentration of diphenylguanidine in the mobile phase. In this experiment, the concentration of diphenylguanidine was optimized in the range from 0.06 to 0.18 mmol/L with an interval of 0.02 mmol/L. By comparing the chromatogram, the response value of the ion-pairing compound increased gradually as the concentration increased. When the concentration was above 0.10 mmol/L, the rate of the response value increased slightly, but the noise was dramatically boosted. From the ultraviolet spectrum (Figure 1), the response value of diphenylguanidine was proportion to the concentration, which was identical to the regularity observed from the chromatogram. For the IPC method, the most suitable response value was 1.5 in the spectrum. In this concentration range, both positive and negative peaks exhibited a broad response range in the chromatogram, and the analytes demonstrated a wide linear range.

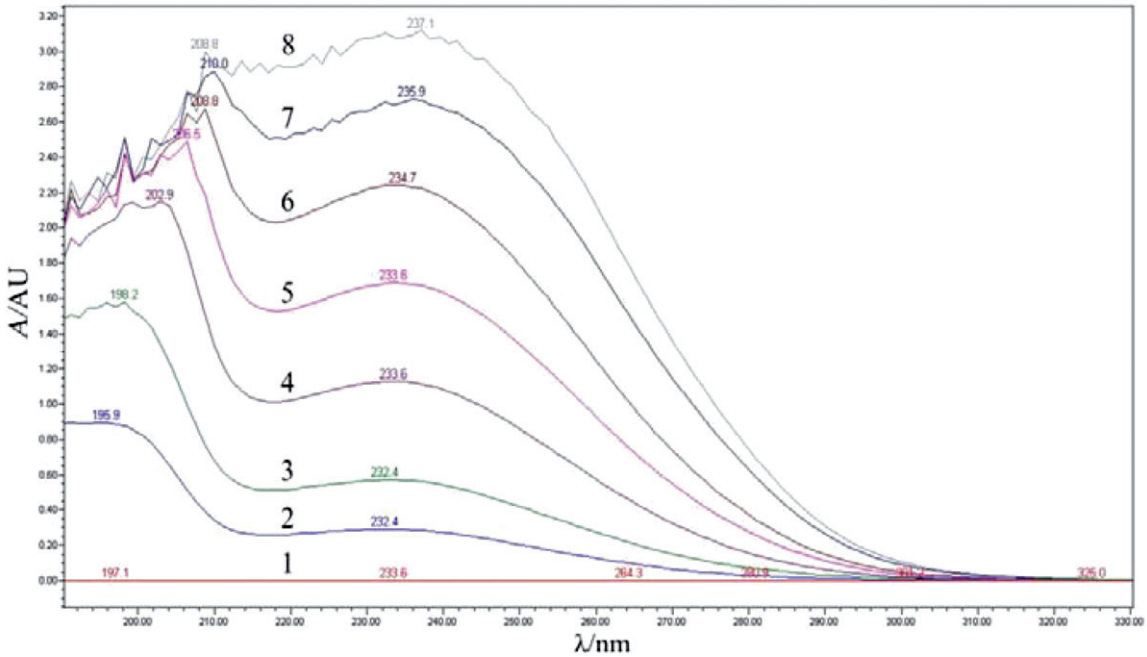

Figure 1. UV spectra of diphenylguanidine at different concentrations (mmol/L): 1. ultrapure water; 2. 0.02; 3. 0.05; 4. 0.10; 5. 0.14; 6. 0.19; 7. 0.24; and 8. 0.28. The diphenylguanidine solution was directly injected into the detector and monitored at wavelengths of 190–400 nm. The spectrum was extracted at 232 nm.

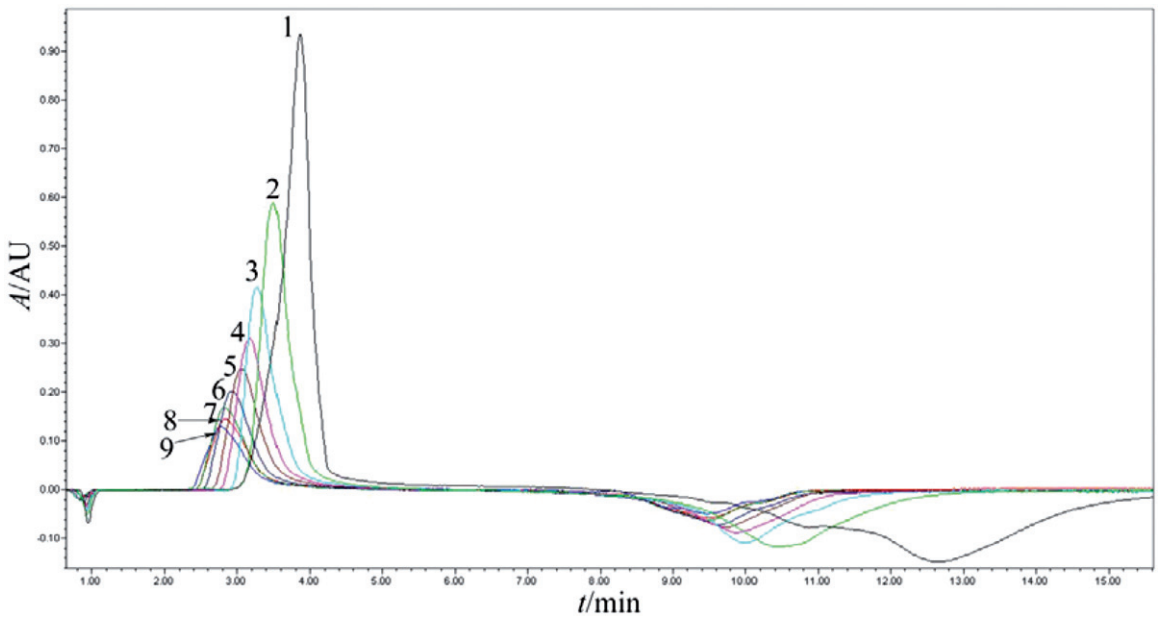

Figure 2. Chromatograms of formic acid modified with *n*-butyric of different concentrations (mmol/L): 1. 0.5; 2. 1.0; 3. 1.5; 4. 2.0; 5. 2.5; 6. 3.0; 7. 3.5; 8. 4.0; and 9. 5.0. A waters SymmetryShield RP_8 chromatographic column as the stationary phase was selected for the separation of formic acid. The mobile phase was the aqueous solution of 0.12 mmol/L diphenylguanidine and the pH value was adjusted with *n*-butyric acid. The flow rate was 1.0 mL/min. The chromatograms were monitored at wavelengths of 190–400 nm and extracted at 232 nm.

Comprehensively considering the response value and signal-to-noise ratio of the ion-pairing compounds, the concentration of diphenylguanidine in the mobile phase was confirmed at 0.12 mmol/L.

Optimization of the modifiers

Both type and concentration of the modifier in the mobile phase affected the generation, retention, and peak shape of the ion-pairing compounds, but the type was more important. Therefore, first, the modifier was chosen from acetic, propionic, *n*-butyric, and *n*-pentanoic acids. The modifiers were fortified into the mobile phase at the same concentration of 2 mmol/L and were optimized for each analyte. By comparing the chromatograms of the same analytes, it can be observed that with increasing the carbon numbers of the modifiers, the retention time of the ion-pairing compound became short, and the response value rose gradually, but the total retention time was prolonged, because the diphenylguanidine progressed out of the column slowly. If the dissociation degree of the modifiers was equal to or

greater than the measured organic acids, the analytes cannot react with diphenylguanidine to form ion-pairing compounds. Therefore, the acidity of the modifier should be weaker than the analyte, that is, the length of carbon chain of the modifiers should be longer than the analytes. According to optimization results, it can be concluded that *n*-butyric acid as a modifier added in the mobile phase was suitable for the analysis of formic, acetic and propionic acid, and *n*-pentanoic acid as a modifier was suitable for *n*-butyric acid.

The next step was to optimize the concentration of the modifier within the range of 0.5–5 mmol/L. From the chromatogram (Figure 2), it can be found that the response value of the ion-pairing compound decreased as the concentration of the modifier increased, and the peak shape of the ion-pairing compound changed from a leading peak to a symmetric peak, and then to a tailing peak. According to the response value and the peak shape, it can be concluded that the modifier of *n*-butyric acid at a concentration of 1.0 mmol/L was suitable for the determination of formic acid, *n*-butyric acid at 0.5 mmol/L was suitable for acetic acid and propionic acid, and the modifier of *n*-pentanoic acid at 1.0 mmol/L was appropriate for the determination of *n*-butyric acid.

In conclusion, the modifier type affected the formation and retention of the ion-pairing compounds and the concentration influenced the response value and peak shapes.

Table 1. Linearity and sensitivity of the saturated monocarboxylic acids.

Analytes	Equation	R^2	LOD/mg L^{-1}	LOQ/mg L^{-1}
Formic acid	$Y = 44554X + 94401$	0.9984	1.0	3.0
Acetic acid	$Y = 15816X + 763542$	0.9963	1.0	3.0
Propionic acid	$Y = 11653X + 532082$	0.9970	5.0	17.0
N-butyric acid	$Y = 10337X + 6957.1$	0.9996	5.0	17.0

Validation of the proposed method

A validation was performed by establishing the calibration curve and obtaining the limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy.

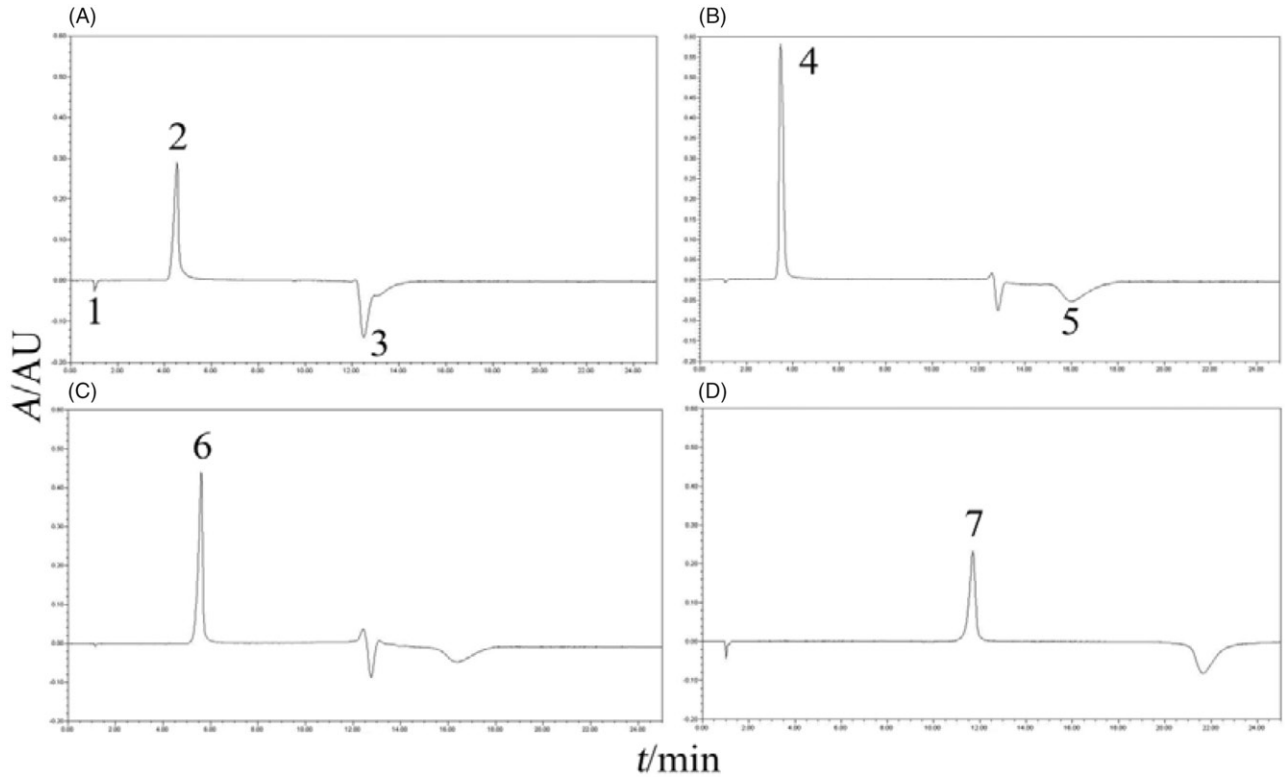

Figure 3. Four typical chromatograms of formic, acetic, propionic, *n*-butyric acids by IPC: A. formic acid; B. acetic acid; C. propionic acid; and D. *n*-butyric acid. The chromatographic peaks: 1. Systemic peak of water; 2. Ion-pairing compound of formic acid; 3. Systemic peak of diphenylguanidine; 4. Ion-pairing compound of acetic acid; 5. Systemic peak of the modifier; 6. Ion-pairing compound of propionic acid; 7. Ion pairing compound of *n*-butyric acid. Chromatographic condition: The Waters SymmetryShield RP_8 chromatographic column was used in the stationary phase. The mobile phase was the aqueous solution of 0.12 mmol/L diphenylguanidine modified with 0.5, 1.0, and 1.0 mmol/L *n*-butyric acid and 1.0 mmol/L *n*-pentanoic acid for the analytes of formic, acetic, propionic, and *n*-butyric acid, respectively. The flow rate was 1.0 mL/min. The chromatograms were monitored at wavelengths of 190–400 nm and extracted at 232 nm.

Table 2. Accuracy and precision of the saturated monocarboxylic acids.

	Low fortification levels			High fortification levels		
Samples	Fortification/mg	Average recovery/%	RSD/%	Fortification/mg	Average recovery/%	RSD/%
Formic acid	10.5	99.8 ± 1.6	1.1	100.5	100.1 ± 0.8	0.51
Acetic acid	20.2	99.9 ± 0.8	0.7	199.0	100.3 ± 0.9	0.61
Propionic acid	20.3	100.7 ± 1.8	1.6	200.2	99.7 ± 1.7	1.1
N-butyric acid	20.2	100.0 ± 0.7	0.51	201.0	98.1 ± 1.7	1.1

Calibration curves were drawn using 11 concentration levels ranging from 10–400 mg/L for formic acid and nine different concentration levels ranging from 50–1400 mg/L for acetic, propionic, and *n*-butyric acids. The linearity curves (area vs. concentration) were plotted for each analyte, and the data were subjected to a regression analysis. The linearity experiments concluded that the peak responses were directly proportional to their concentrations (Table 1). The linear relationships of the four kinds of acids all performed well and the correlation coefficients (R^2) were all above 0.996. Typical chromatograms of the four kinds of acids are shown in Figure 3.

The LOD and LOQ for each analyte were determined at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of standard solutions with known concentrations. The LOD of formic acid and acetic acid was 1.0 mg/L, the LOD of propionic and *n*-butyric acid was 5.0 mg/L, and the LOQs corresponding to the sample of analytes were 3.0 and 17.0 mg/L (Table 1).

The accuracy and precision of the macro-analysis were assessed by determining the content of analyte in the solution, which was prepared by adding the same amount of standard samples into the sample solution. The recovery and relative standard deviation were computed. The fortification amounts of formic acid were 0.01 g and 0.1 g, and the amounts for other acids were 0.02 g and 0.2 g. Each fortification level was repeated 5 times. The high fortification levels of the four kinds of acids were all diluted 10-fold before injection into the system. The accuracy and precision results of the four kinds of acids are listed in Table 2. The average recovery of the high and low fortification levels was between 98.1% and 100.7%, which is close to 100% in all cases. The RSD values of the four kinds of acid samples were all below 2%.

Determination of the sample content

The samples of different acids were separately determined by the IPC and titrimetric method. The results are presented in Table 3. The sample contents of acetic acid obtained by the two methods were similar to each other. The maximum difference was merely 1.9%. This difference may be attributed to its special property to easily produce a high-purity reagent with less impurities. The results of the formic and propionic acids exhibited a slightly greater difference between the two methods: the difference for formic acid was 5.6%, and the difference for propionic acid was 2.9%. The results acquired by the titration method were all higher than those of the IPC method, which may be attributed to the impurities in the samples consuming the basic titrant. However, the IPC method can avoid this interference by separating the analytes with impurities, so that the measured results are more reliable.

Table 3. Sample results of formic acid, acetic acid, propionic acid measured by the IPC and titration method.

Samples	IPC/%	Titration/%	Average absolute deviation/%
Formic acid	80.6	86.2	5.6
Acetic acid 1#	98.0	99.8	1.9
Acetic acid 2#	99.6	99.7	0.2
Acetic acid 3#	91.2	91.4	0.3
Propionic acid 1#	96.9	99.8	2.9

Standard additive method for the acid mixtures

The IPC method was not suitable for calibrating mixtures of organic acids with the single standard solution prepared above. Because each acid has a different degree of dissociation, the stronger acids inhibited the dissociation of the weaker acids in the solution of the mixture, which led to a measured content of the weak acid less than the real value. To solve this problem, a standard additive method was adopted to eliminate the background difference between the standard solution and the mixture.

To simultaneously analyze the four kinds of acid and to ensure the high response value, an aqueous solution of 0.12 mmol/L diphenylguanidine/1.0 mmol/L n-pentanoic acid was prepared as the mobile phase. The four kinds of acid were separated completely. The retention times of formic, acetic, propionic and *n*-butyric acid were 3.52, 2.94, 5.23, and 11.76 min, respectively. Within the concentration range of 50–1000 mg/L, the linear regression equation of propionic acid was $Y = 45974 + 301.31\ X$, the linear regression equation of *n*-butyric acid was $Y = -25071 + 371.28\ X$, and all the correlation coefficients (R^2) were above 0.999. Although the linear relationship of the two acids in the mixed solutions performed well, the propionic and *n*-butyric acids were still inhibited by the stronger acids, namely, formic and acetic acid. The amount of dissociated ions decreased and the response value of ion-pairing compounds was dramatically lower than that of the single standard solution of the same concentration. This phenomenon illustrated that the weak acid was inhibited by the strong acid from another perspective. The recovery of propionic acid in the mixture was between 99.3% and 100.6%, and the recovery of *n*-butyric was between 98.5% and 101.4%, and these results

Table 4. Results of propionic acid and n-butyric acid measured by the standard additive method.

	Compound concentration/mg L^{-1}				Measured value/ mg L^{-1}		Recovery/%		Average recovery/%	
No.	Formic acid	Acetic acid	Propionic acid	*N*-butyric acid	Propionic acid	*N*-butyric acid	Propionic acid	*N*-butyric acid	Propionic acid	*N*-butyric acid
1#	10.1	102.5	152.4	197.8	151.8	199.8	99.6	101.0	100.0	99.6
2#	20.2	153.8	101.6	98.9	100.9	97.4	99.3	98.5		
3#	50.4	102.5	203.1	148.4	204.0	146.8	100.4	98.9		
4#	100.8	205.1	203.1	98.9	202.8	100.3	99.8	101.4		
5#	151.2	205.1	203.1	203.1	204.4	199.0	100.6	98.0		

are listed in Table 4. Although the concentrations of the strong acids (e.g. formic acid) in the background were different between the standard and sample solutions, this difference exhibited little effect on the results. It can be concluded that the matrix effect can be eliminated by adding a certain amount of the sample into the standard solution, which ensured that the results were close to the real value.

Conclusion

The IPC method for the determination of formic, acetic, propionic, and *n*-butyric acid by HPLC-DAD was established. Delicate relationships were demonstrated among the ion-pairing reagent, modifiers and the analytes. Although the ion-pairing reagent – diphenylguanidine – can react with analytes to form ion-pairing compounds without adding modifiers into the mobile phase, the retention time of the ion-pairing compound was short and the shape of chromatographic peak was wide. The modifiers affected the formation of the ion-pairing compounds and played an important role in adjusting the response value and peak shape of the ion-pairing compounds. The length of the carbon chain of the modifier needs to be longer than that of the analytes. The established method demonstrated a broad linear range, high precision and good accuracy for analysis of macro-organic acids. The IPC method was more accurate than the titrimetric method in determining the content of the analytes. A standard additive method was suitable for determining the content of acids in the mixtures. Our proposed method was an accurate, reliable, and efficient method for analysis of the four kinds of acids. The application of diphenylguanidine to other nonconjugate organic anionic compounds, such as pentandioic acid, itaconic acid, alendronate sodium, and pamidronate disodium, is worth considering.

Disclosure statement

No potential conflict of interest was reported by the authors.

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