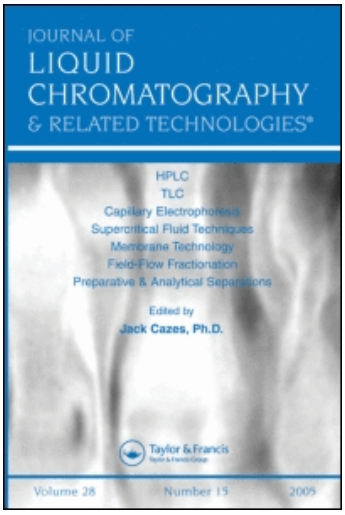

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF α-SUBSTITUTED ACETIC ACID DERIVATIVES

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ABSTRACT

Two high-performance liquid chromatographic systems for the separation of α-substituted acetic acid derivatives are presented. The first method uses a resin-based column for organic acid separations (Polypore H) with a dilute acid as mobile phase. The second system decribes the possibilities of ion-pair high-performance liquid chromatography on a reverse phase C18 column. Special attention is given to the simultaneous optimization of the counterion and buffer concentration. The applicability is demonstrated in the quality control of [1-^{11}C]-malonic acid.

INTRODUCTION

Malonic acid is frequently used for the synthesis of pharmaceuticals. As a consequence, both carbon-

14 and carbon-11 labeled malonic acid are interesting precursors for the production of corresponding radiopharmaceuticals to perform extended biomedical studies. For this purpose, malonic acid is mostly prepared by the nitrile synthesis :

$$X\text{-}CH_2\text{-}COOH + {}^{-}CN \rightarrow NC\text{-}CH_2\text{-}COOH \xrightarrow{H_2O} HOOC\text{-}CH_2\text{-}COOH$$

where -X is a good leaving group (-Cl, -Br, -I). By nucleophilic displacement reaction, the haloacetate reacts with cyanide to form cyanoacetate, which is then converted to malonic acid by an acid or base catalysed hydrolysis (1,2).

The optimization of the synthesis process requires a selective analytical method, which should also be rapid in the case of the carbon-11 labeled malonic acid synthesis because of the short half-life of the isotope ($t_{1/2}$ = 20.4 min) (3).

As they have quite similar properties, e.g. polarity, pK_a, solubility in water, the separation of the starting compound (haloacetic acid), the intermediate compound (cyanoacetic acid), the final product (malonic acid) and the main side product (hydroxyacetic acid) is an interesting problem. Recently, we described an ion-pair reverse phase

HPLC determination of malonic acid in the presence of acetic acid (4). The aim of the present study was to evaluate both the possibilities of a resin-based column (Polypore H) and ion-pair reverse phase HPLC for the separation of α-substituted acetic acid derivatives. The influence of buffer and counterion concentration as optimization variables in the ion-pair system were studied in more detail. In addition, the applicability of the described system is demonstrated in the radiochemical purity determination of [1-^{11}C]-malonic acid.

MATERIALS AND METHODS

Apparatus and Columns

A Waters M510 pump was used in combination with a Pye Unicam LC3 variable wavelength uv absorbance detector and a Valco sixport switching valve. Chromatograms were recorded on a LKB 2210 dual pen recorder. For the radiochemical experiments, a NaI(Tl) detector was placed on line after the uv detector.

Chromatography was performed with a Polypore H column (220x4.6mm i.d.) (Pierce, U.S.A.) with 10 micron particles and a Lichrosorb RP C18 column

(150x4.6mm i.d.) packed with 5 micron material (Alltech, Belgium).

Chemicals

Analytical-grade chloroacetic acid, bromoacetic acid, iodoacetic acid and malonic acid were obtained from Janssen Chimica (Beerse, Belgium) ; hydroxyacetic acid, sodium cyanide, sulphuric acid, phosphoric acid, formic acid, sodium hydroxide and sodiumdihydrogen phosphate were obtained from UCB (Leuven, Belgium) and tetrabutylammonium hydrogen sulphate (TBA) from Aldrich (Brussels, Belgium).

Double-distilled water was used in eluents and standard solutions. Eluents were degassed in an ultrasonic bath prior to use.

Procedures

Standard solutions (0.5 mg/mL) of the compounds were prepared by weighing and dissolving in water. The uv detector was set at 220 nm and the temperature was ambient (21 $\pm$ 2 °C). A sample loop of 20 µL was used. The flow rate was 0.3 mL/min for the Polypore H column and 1.5 mL/min for the Lichrosorb column. Mobile phase compositions are reported under Results and Discussion.

RESULTS AND DISCUSSION

The separation mechanism on Polypore H is a combination of ion exclusion, partition and ligand exchange. As the mobile phase has to be a dilute acid, changes in selectivity can be obtained by a limited number of variables : temperature, acid concentration and, most important, choice of the acid. Three different acids were tried. The data are given in Table 1. The column dead time t_o was determined by the first baseline deflection.

Phosphoric and sulphuric acid gave similar results while formic acid showed a decreased overall selectivity.

Figure 1 shows the separation between cyano- and chloroacetic acid with sulphuric acid as mobile phase. Sodium cyanide showed no retention, as was proved by injecting C-14-labeled sodium cyanide, followed by fractionation of the column eluate and subsequent liquid scintillation counting. A disadvantage of these systems is the incompatibility of the stationary phase with cations, which can partly be set off by the use of guard cartridges. For samples with high salt concentration, e.g. synthesis residues, a preliminary purification step is thus required.

TABLE 1

Capacity factors (k') obtained with a Polypore H column

	Mobile phase		
	H_2SO_4 (0.005 M)	H_3PO_4 (0.005 M)	HCOOH (0.01.M)
Cl-CH_2-COOH	1.37	1.34	1.35
Br-CH_2-COOH	1.74	1.65	1.26
I-CH_2-COOH	1.97	1.82	1.39
NC-CH_2-COOH	0.89	0.95[1]	0.72[1d]
HOOC-CH_2-COOH	0.58	0.51	0.37
HO-CH_2-COOH	0.90	0.92	0.86

1 : leading peak ; d : peak doubling
t_o = 3.57 minutes

Further more, some compounds of interest, e.g. cyano- and hydroxyacetic acid, are not well separated.

With regard to these aspects, ion-pair reverse phase HPLC is a more flexible system. Most separations are optimized by varying pH, choice and concentration of both the counterion and organic modifier. Generally, very little attention has been paid to the buffer. However, as a consequence of the multiple

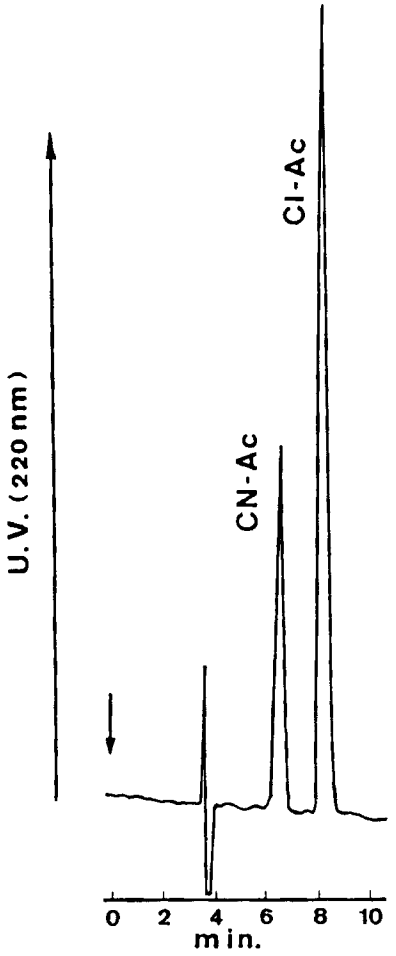

FIGURE 1. Chromatogram showing the separation between cyanoacetic acid (CN-Ac) and chloroacetic acid (Cl-Ac). A Polypore H column with dilute sulphuric acid as mobile phase was used.

equilibria and complexes formed between sample molecules, counterions, buffer species, corresponding co-ions and stationary phase groups, one can expect the buffer to play a significant role in a chromatographic system. Indeed, Melander et al. explored the use of different acidic amine phosphate buffers for the separation of ionogenic substances on non polar stationary phases (5).

Under the conditions we used, i.e. the same column (phase ratio and C18 ligand concentration), pH (6.5) and species of the mobile phase (equilibrium distribution constants), k' is dependent on the sodiumphosphate buffer and TBA counterion concentration. The experimental conditions are visualized in Figure 2. The experimental design is based on a two-level factorial system (6) and the experimental region has been choosen on the basis of previous experience (4). For each of the compounds, the total experimental region (experiment number 1 to 5) is thus devided into four planes, with the k' value as the response. The results are shown in Table 2.

Significant changes in capacity factors can be obtained by the simultaneous change of sodiumphosphate and TBA concentration. With all the

TABLE 2

Capacity factors (k') obtained with ion-pair reverse phase HPLC

	Experiment number						
	1	2	3	4	5	6	7
Cl-CH_2-COOH	7.1	2.1	3.1	4.3	3.8	4.6	6.8
Br-CH_2-COOH	8.0	3.1	4.6	6.3	5.5	6.4	8.1
I-CH_2-COOH	11.2	5.0	6.8	9.4	8.0	9.8	11.4
NC-CH_2-COOH	5.0	1.4	2.0	2.9	2.5	3.0	4.5
HOOC-CH_2-COOH	11.3	1.1	1.5	4.1	3.1	4.7	15.5
HO-CH_2-COOH	1.8	0.5	0.6	1.0	0.9	1.1	2.5

acids, increasing the buffer concentration does decrease the k' value. The magnitude of this effect however is strongly dependent on the TBA concentration, indicating an interaction between the two variables. Clearly, a one-factor-at-a-time approach would not yield valid results. The influence of the counterion concentration is also dependent on the buffer concentration level, not only with regard to the magnitude, but also to the sign of the effect. At high buffer concentration, retention

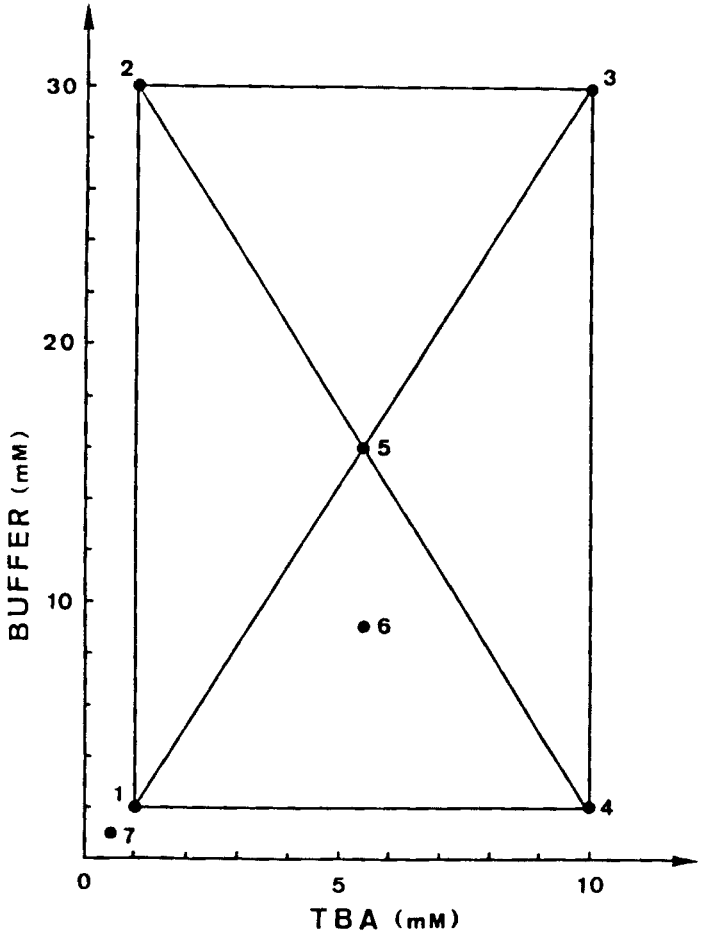

FIGURE 2. Representation of the different levels of the two variables in the experiments.

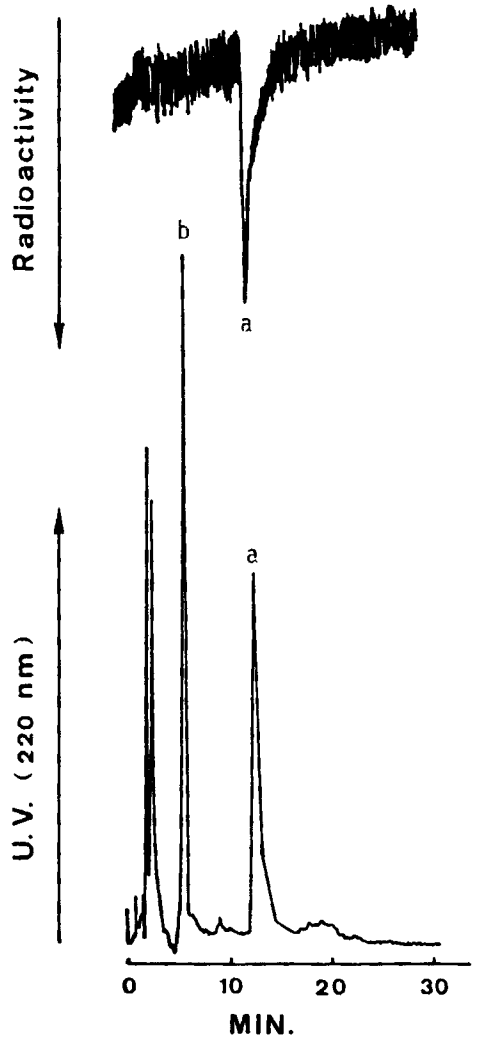

FIGURE 3. Quality control chromatogram of 1-[^{11}C]-malonic acid using ion-pair RP HPLC. Stationary phase: LiChrosorb RP C18, 5 µm, 150x4.6 mm ; mobile phase : 1 mM sodium-phosphate/0.5 mM TBA, adjusted to pH 6.5; flow rate : 2 mL/min ; detector : radio-activity : NaI(Tl)-[log] and uv at 220 nm. Peaks are malonic acid (a) and hydroxyacetic acid (b).

is increased with higher TBA concentration, while the opposite is observed with low buffer concentration. From this, it is clear that complex formation in the mobile phase and adsorption of the counterion to the stationary phase are both important and influenced by the buffer ions. Furthermore, the residual silanol groups may also play a role (5).

The ion-pair reserve phase system was used to optimize the [1-^{11}C]-malonic acid synthesis. Figure 3 is a typical quality control chromatogram obtained from the synthesis residue. In this way, the chemical and radiochemical purity could be demonstrated.

CONCLUSION

Not all α-substituted acetic acid derivates are separated on the Polypore H column. Furthermore applications are limited to samples containing little or no cations. On the other hand, the optimized ion-pair HPLC system on a reverse phase C18 column gave good results. The optimization was performed by simultaneous variation of the buffer and counterion concentration. In this way, we demonstrated the influence and interaction of these two factors.

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