

## Degradation kinetics of benzonatate in aqueous solutions

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### Abstract

The decomposition of benzonatate in aqueous solutions followed apparent first-order kinetics. One major hydrolysis product, 4-(butylamino)benzoic acid, was efficiently and completely separated from benzonatate with a validated HPLC. The apparent activation energies obtained from an Arrhenius plot was 16.07, 20.54 and 18.23 kcal mol<sup>-1</sup> in buffer solutions with pH 3.61, 9.42 and 10.46, respectively, which indicated that hydrolysis dominated the degradation process. The buffer concentration showed significant effect on the hydrolysis of benzonatate ( $P < 0.05$ ). Specific acid-, specific base- and buffer catalyzed hydrolysis of benzonatate were observed in solutions with pH in the range of 0.31–12.21. The decomposition of benzonatate in basic solutions was faster than in acidic solutions by about 2000-fold. The prominent base-catalyzed breakdown of the ester bond and slower acid-catalyzed hydrolysis suggested that benzonatate should be prepared in the solutions with pH 3–7 to maximize its stability. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Benzonatate; Hydrolysis; Temperature; pH; Buffer

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### 1. Introduction

Benzonatate is a non-narcotic oral antitussive agent with local anesthetic properties that is chemically related to tetracaine and other topical anesthetics (Simon, 1957). It is used clinically for the symptomatic relief of coughing and may also be effective in suppressing coughing associated with acute and chronic respiratory diseases (Gre-

goire et al., 1958; Cohan et al., 1986). It has been reported that the onset of its therapeutic effects occurs within 15–20 min and the duration of the antitussive action is maintained for 3–8 h (Gre-goire et al., 1958; Eddy et al., 1969).

Several references related to the pharmacology and mechanism of benzonatate action have been reported (Michelson and Schiller, 1957; Wilson et al., 1958; Tomokazu, 1967). However, relevant pharmacokinetic data are limited. The rapid hydrolysis of benzonatate into 4-(butylamino)benzoic acid by pseudocholinesterase in

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vivo (Wetherell and French, 1986) and the lack of a proper analytical method to determine the concentration of benzonatate in plasma limited the corresponding pharmacokinetic studies (Cohan et al., 1986; Kelly et al., 1993).

The ester linkage in benzonatate chemical structure (Fig. 1) is liable to hydrolysis. However, data concerning the chemical stability of benzonatate in aqueous solutions is limited. The better understanding of the chemical stability of benzonatate under various conditions will assist the development of other proper formulations as well as the proper storage condition. The objective of this study was to investigate the hydrolytic kinetics of benzonatate in aqueous solutions. The kinetic studies were conducted at various temperatures, buffer concentrations and pH. The possible decomposition products and pathways were discussed.

## 2. Materials and methods

### 2.1. Materials

Benzonatate (lot # P00080) were donated by Lu Ren Company (Gau Shiung, Taiwan). 4-(Butylamino)benzoic acid was from Aldrich Chemical Company (Milwaukee, WI). Phosphoric acid and tetrabutylammonium hydrogensulfate were from Merck (Darmstadt, Germany). Sodium pyrosulfite was from Wako Pure Chemical Industries, LTD (Osaka, Japan). HPLC grade acetonitrile and methanol were from Fisher Scientific International Company (Leicestershire, UK).

### 2.2. HPLC conditions

The HPLC analytical method applied here was according to the previous report (Lin et al., 1996). The HPLC was equipped with a Merck LiChrosorb RP-8 column ( $4 \times 250$  mm,  $7 \mu\text{m}$ ), a



Fig. 1. The chemical structure of benzonatate.

SHIMADZU LC-10AT solvent delivery system, a JASCO WIDEC-100-V detector at 310 nm and a SHIMADZU C-R6A chromatorecorder. The mobile phase was a mixture of acetonitrile: methanol: 0.05 M phosphoric acid (pH 3.0) = 42:10:48% (v/v) while 1% (w/v) tetrabutylamine hydrogensulfate was added. The flow rate was 1.3 ml min<sup>-1</sup>. Six standard solutions with concentrations ranging from 2–40 to 0.2–12 µg ml<sup>-1</sup>, respectively, were prepared to construct the calibration curves for benzonatate and 4-(butylamino)benzoic acid. The intra- and inter-day precision of the HPLC analytical method were determined. The concentrations representing the entire range of the calibration curve were studied with six replica within 1 day and once daily for 6 days for each concentration. The mean, standard deviation (SD) and coefficient of variance (CV%) were determined.

### 2.3. Preparation of buffer and unbuffered solutions

A total of 20 buffer solutions with ionic strength 0.3 in the pH range of 3.61–11.50 and seven unbuffered solutions were prepared according to Table 1. The pH of each prepared solution was determined with a pH meter (Mettler 320). Benzonatate was added into each solution and the concentrations of benzonatate and its major degradation product, 4-(butylamino)benzoic acid, were determined with a validated HPLC. The hydrolysis rate constants of benzonatate were calculated based on a proper kinetic model.

### 2.4. Influence of buffer concentration

The degradation of benzonatate in aqueous solutions were further studied in two acetate buffer solutions (pH 3.61 and 4.38) and three carbonate buffer solutions (pH 9.42, 9.95 and 10.46) with various buffer concentrations, 0.025, 0.05 and 0.1 M, at 60°C. The triplicate apparent rate constants ( $n = 3$ ) from solutions with three different buffer concentrations were analyzed by one-way ANOVA ( $\alpha = 0.05$ ) to test whether the variation appeared in apparent hydrolysis rate

Table 1  
Properties of 27 buffer and unbuffered solutions

No.	pH ( $\pm 0.05$ )	Species	Conc. (M)	Capacity ( $\times 10^3$ )	$k_{\text{obs}} \times 10^6 \text{ (min}^{-1}\text{)}$		
					60°C	50°C	70°C
1	0.31	HCl	0.5	1127.96	392.50		
2	0.55	HCl	0.25	649.07	188.00		
3	1.34	HCl	0.05	105.27	32.59		
4	1.68	HCl	0.025	48.12	14.53		
5	2.63	HCl	0.0025	5.40	2.44		
6	3.61	Acetate	0.025	3.72	1.42	1.20	5.23
7	3.61	Acetate	0.05	7.44	2.30		
8	3.61	Acetate	0.1	14.88	2.36		
9	4.38	Acetate	0.025	11.97	0.86	0.99	2.49
10	4.38	Acetate	0.05	23.94	1.06		
11	4.38	Acetate	0.1	47.88	1.28		
12	5.42	Acetate	0.025	8.43	0.52		
13	6.47	Phosphate	0.025	7.63	0.94		
14	7.40	Phosphate	0.025	13.62	5.57		
15	8.60	Carbonate	0.025	1.04	57.60		
16	9.42	Carbonate	0.025	5.63	263.83 <sup>a</sup>	111.54 <sup>a</sup>	721.50 <sup>a</sup>
17	9.42	Carbonate	0.05	11.26	265.83 <sup>a</sup>		
18	9.42	Carbonate	0.1	22.52	287.50 <sup>a</sup>		
19	9.95	Carbonate	0.025	11.96	661.00 <sup>a</sup>		
20	9.95	Carbonate	0.05	23.93	758.83 <sup>a</sup>		
21	9.95	Carbonate	0.1	47.86	854.33 <sup>a</sup>		
22	10.46	Carbonate	0.025	14.05	2098.33 <sup>a</sup>	876.00 <sup>a</sup>	4596.67 <sup>a</sup>
23	10.46	Carbonate	0.05	28.10	2618.33 <sup>a</sup>		
24	10.46	Carbonate	0.1	56.20	3321.67 <sup>a</sup>		
25	11.50	Phosphate	0.025	6.61	12272.00 <sup>a</sup>		
26	11.50	NaOH	0.01	7.28	5624.38		
27	12.21	NaOH	0.025	37.30	28088.19		

<sup>a</sup> Three runs were performed and the mean value was indicated.

constants was attributed from the concentration of buffer.

### 2.5. Influence of pH and buffer

The degradation of benzonatate were studied in nine buffer solutions with ionic strength 0.3, buffer concentration 0.025 M and pH in the range of 3.61–11.50 at 60°C. Five unbuffered HCl solutions with pH in the range of 0.31–2.63 as well as two unbuffered NaOH solutions with pH 11.50 and 12.21 were also included in this study. The concentration of benzonatate remained in each solution was then plotted as a function of time on the semi-logarithmic paper and the apparent hydrolysis rate constant was obtained from the slope.

### 2.6. Determination of dissociation constant of benzonatate

The dissociation constant of benzonatate was determined by a nonlogarithmic linear titration method (Hofstee, 1960; Benet and Goyan, 1965). Benzonatate was accurately weighed (50 mg) and dissolved in 50 ml of freshly deionized water. The titration was performed at 26.0 ± 0.5°C. Small volume of 0.01 N sodium hydroxide was added with a micropipette. The solution was then mixed well with a magnetic stirrer and the pH value of solution following each titration was measured with a pH meter (Mettler Toledo 320). Three runs were performed. The data were treated by plotting the concentration of unionized benzonatate, [B], versus the product of [B] and [H<sup>+</sup>] and the slope

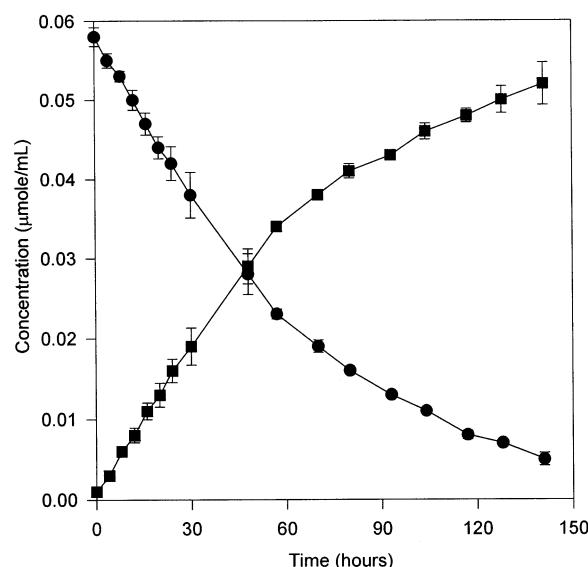


Fig. 2. A kinetic plot of the hydrolysis of benzonatate and the formation of 4-(butylamino)benzoic acid in a 0.025 M carbonate buffer solution with ionic strength 0.3 and pH 9.42 at 60°C. (●) benzonatate, (■) 4-(butylamino)benzoic acid.

of the linear portion corresponded to the reciprocal of the dissociation constant.

### 3. Results and discussion

#### 3.1. HPLC condition

The HPLC analytical method applied to this study was validated. The retention time of benzonatate and 4-(butylamino)benzoic acid were 5.2 and 3.9 min, respectively and both peaks were completely separated. The calibration curves were linear with the correlation coefficients greater

Table 2  
Activation energies, the frequency factors,  $k_{25^\circ\text{C}}$  and the shelf-life ( $t_{90}$ ) for benzonatate hydrolysis at various pH values

pH	Ea (kcal mol <sup>-1</sup> )	A (min <sup>-1</sup> )	$k_{25^\circ\text{C}}$ (min <sup>-1</sup> )	$t_{90}$ (h)
3.61	16.07	$7.31 \times 10^4$	$0.12 \times 10^{-6}$	14494.79
9.42	20.54	$8.25 \times 10^9$	$7.23 \times 10^{-6}$	242.97
10.46	18.23	$1.77 \times 10^9$	$76.55 \times 10^{-6}$	22.94

Table 3

Apparent rate constants of benzonatate in buffer solutions with ionic strength 0.3 in five pH values at 60°C

pH	Buffer conc. (M)	$k_{\text{obs}} \times 10^6$ (min <sup>-1</sup> )
3.61	0.025	1.42
	0.05	2.29
	0.1	2.36
	0	1.39 <sup>a</sup>
4.38	0.025	0.86
	0.05	1.06
	0.1	1.28
	0	0.75 <sup>a</sup>
9.42	0.025	$263.83 \pm 7.27^b$
	0.05	$265.83 \pm 9.34^b$
	0.1	$287.50 \pm 6.80^b$
	0	253.00 <sup>a</sup>
9.95	0.025	661.00
	0.05	758.83
	0.1	854.33
	0	613.25 <sup>a</sup>
10.46	0.025	$2098.33 \pm 18.75^b$
	0.05	$2618.33 \pm 26.13^b$
	0.1	$3321.67 \pm 53.89^b$
	0	1746.67 <sup>a</sup>

<sup>a</sup> The apparent rate constants were obtained from extrapolation to zero buffer concentration.

<sup>b</sup> Three runs were performed and the mean  $\pm$  SD was indicated.

than 0.999 over the working range of concentrations for both compounds. Both coefficients of variation of intra- and inter-day precision were less than 3.5% for benzonatate and less than 7.5% for 4-(butylamino)benzoic acid. The reproducibility of the analytical method was determined and the mean concentrations were within 5.0 and 10.0% of the actual values of benzonatate and 4-(butylamino)benzoic acid, respectively.

#### 3.2. Order of reactions

A kinetic plot of the hydrolysis of benzonatate and the formation of 4-(butylamino)benzoic acid at 60°C in a 0.025 M carbonate buffer solution with ionic strength 0.3 and pH 9.42 is shown in Fig. 2. The decrease in the concentration of benzonatate and the increase in the concentration of 4-(butylamino)benzoic acid followed the mass bal-

ance theory. This result indicated that no further decomposition of 4-(butylamino)benzoic acid was undergone in this storage condition. The decomposition of benzonate in aqueous solution followed pseudo first-order kinetics, which was indicated by a good linearity ( $r^2 > 0.99$ ) of plot of the concentration of benzonate against time on the semi-logarithmic paper. The mean apparent rate constant calculated by following the decrease in the concentration of benzonate with time was  $(2.64 \pm 0.09) \times 10^{-4} \text{ min}^{-1}$ . The mean apparent rate constant determined by following the increase in the concentration of 4-(butylamino)benzoic acid with time was  $(2.57 \pm 0.26) \times 10^{-4} \text{ min}^{-1}$ . Both values were close to each other.

### 3.3. Influence of temperature

The activation energies and frequency factors obtained from the degradation of benzonate in buffer solutions with pH values 3.61, 9.42 and 10.46 at 50, 60 and 70°C are listed in Table 2. The values of the activation energy were 16.07, 20.54 and 18.23 kcal mol<sup>-1</sup> in buffer solutions with pH 3.61, 9.42 and 10.46, respectively. The reported activation energies for the hydrolysis of a few

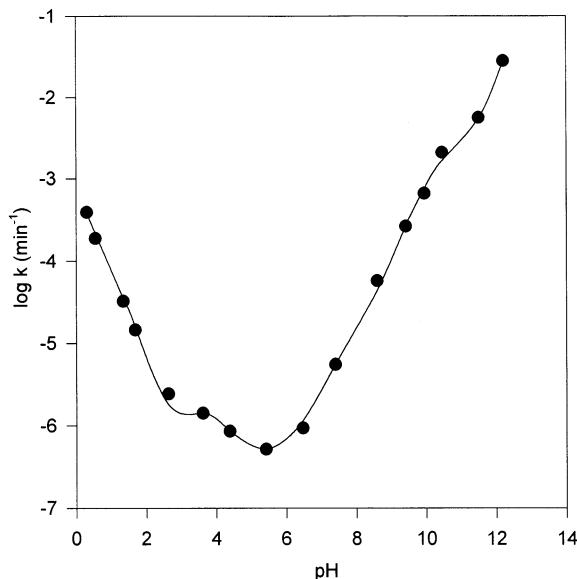


Fig. 3. The plot of logarithmic values of apparent hydrolysis rate constants ( $k_{\text{obs}}$ ) as a function of pH. The curve was fitted by Eq. (6).

compounds ranged from 14 to 23 kcal mol<sup>-1</sup> (Cannors et al., 1986). The values obtained from this study were within the reported values, which suggested that the hydrolysis process dominated the decomposition of benzonate in solutions. If the degradation mechanism in the interested temperature range was the same, then the activation energies and the frequency factors listed in Table 2 could be further applied to predict the theoretical shelf-life of benzonate in the solution under the specific pH and temperature. The theoretical rate constant at 25°C ( $k_{25^\circ\text{C}}$ ) and the shelf-life ( $t_{90}$ ) were calculated and listed in Table 2. The time for 10% of benzonate to be decomposed was 14494.79 h in the pH 3.61 acidic solution and 242.97 h in the pH 9.42 basic solution. However, 10% of benzonate was hydrolyzed within 22.94 h in the pH 10.46 basic solution.

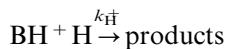
### 3.4. Influence of buffer concentration

The buffer concentrations and the corresponding apparent rate constants in five pH values are shown in Table 3. The hydrolysis rate of benzonate was much faster in carbonate buffer solutions than in acetate buffer solutions, and the significant effect of carbonate concentration on the hydrolysis rate of benzonate was observed in aqueous solutions with pH 9.42 and 10.46 ( $P < 0.05$ ). For each pH value, a plot of  $k_{\text{obs}}$  against buffer concentration yielded a graph with an intercept corresponding to the observed rate constant at zero buffer concentration. These values are summarized in Table 3.

### 3.5. Influence of pH and buffer

The catalytic effects of buffer components and the influence of pH on benzonate hydrolysis in aqueous solutions were examined in nine buffer solutions and seven unbuffered solutions with pH values ranging from 0.31 to 12.46 at 60°C. In order to elucidate the catalytic effect on benzonate hydrolysis, the  $pK_a$  of benzonate was determined with a titration method and the determined value was  $4.91 \pm 0.12$ . In the pH range of 0.31–2.63, only HCl was presented in the solution. Since the  $pK_a$  of benzonate is 4.91, in this pH

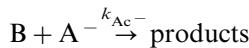
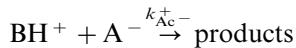
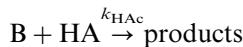
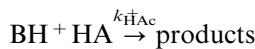
region only protonated benzonatate was predominant and the major reaction undergone can be expressed as:



The apparent rate constant in this pH range can be written as Eq. (1).

$$k_{\text{obs}} = k_{H^+}^+ [H^+] \quad (1)$$

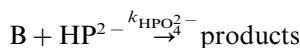
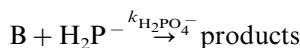
In the pH range of 3.61–5.42, since the  $pK_a$  value of acetic acid is 4.76 (Skoog et al., 1992), the acetate buffer consisted both  $[CH_3COOH]$  and  $[CH_3COO^-]$  and both species could be considered to be the catalytic species. In this pH region both ionized and unionized benzonatate were presented and the major reactions undergone can be expressed as:



The apparent rate constant can be written as Eq. (2).

$$k_{\text{obs}} = k_{HAc}^+ [HAc] \frac{[H^+]}{[H^+] + K_a} + k_{HAc} [HAc] \frac{K_a}{[H^+] + K_a} + k_{Ac^-}^+ [Ac^-] \frac{[H^+]}{[H^+] + K_a} + k_{Ac^-} [Ac^-] \frac{K_a}{[H^+] + K_a} \quad (2)$$

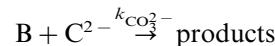
In the pH range of 6.47–7.40, the  $pK_{a2}$  value of phosphoric acid is 7.20 (Skoog et al., 1992), the phosphate buffer consisted both  $[H_2PO_4^-]$  and  $[HPO_4^{2-}]$  and both ions could be considered to be the catalytic species. Since in this pH region unionized benzonatate was predominant and the major reactions undergone can be expressed as:



The apparent rate constant can be written as Eq. (3).

$$k_{\text{obs}} = k_{H_2PO_4^-} [H_2PO_4^-] + k_{HPO_4^{2-}} [HPO_4^{2-}] \quad (3)$$

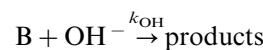
In the pH range of 8.60–10.46, because the  $pK_{a2}$  value of carbonic acid is 10.33 (Skoog et al., 1992), the carbonate buffer consisted both  $[HCO_3^-]$  and  $[CO_3^{2-}]$  and both ions could be considered to be the catalytic species. Since in this pH region unionized benzonatate was predominant and the major reactions undergone can be expressed as:



The apparent rate constant can be written as Eq. (4).

$$k_{\text{obs}} = k_{HCO_3^-} [HCO_3^-] + k_{CO_3^{2-}} [CO_3^{2-}] \quad (4)$$

In the pH range of 11.50–12.21, only NaOH was presented in the solution. Since the unionized form of benzonatate was predominant, the major reaction undergone can be expressed as:



The apparent rate constant in this pH range can be written as Eq. (5).

$$k_{\text{obs}} = k_{OH^-} \frac{k_w}{[H^+]} \quad (5)$$

Therefore, in the pH range of 0.31–12.21, the Eqs. (1)–(5) were combined and Eq. (6) was obtained.

$$k_{\text{obs}} = K_H^+ [H^+] + k_{HAc}^+ [HAc] \frac{[H^+]}{[H^+] + K_a} + k_{HAc} [HAc] \frac{K_a}{[H^+] + K_a} + k_{Ac^-}^+ [Ac^-] \frac{[H^+]}{[H^+] + K_a} + k_{Ac^-} [Ac^-] \frac{K_a}{[H^+] + K_a} + k_{H_2PO_4^-} [H_2PO_4^-] + k_{HPO_4^{2-}} [HPO_4^{2-}] + k_{HCO_3^-} [HCO_3^-] + k_{CO_3^{2-}} [CO_3^{2-}] + k_{OH^-} \frac{k_w}{[H^+]} \quad (6)$$

where:  $k_H^+$ , second-order rate constant for specific acid-catalysis of protonated benzonatate;  $k_{HAc}^+$ , second-order rate constant for acetic acid catalysis of protonated benzonatate;  $k_{HAc}$ , second-order rate constant for acetic acid catalysis of unionized benzonatate;  $k_{Ac^-}^+$ , second-order rate constant for acetate catalysis of protonated benzonatate;  $k_{Ac^-}$ , second-order rate constant for acetate catalysis of unionized benzonatate;  $k_{H_2PO_4^-}$ , second-order rate constant for  $H_2PO_4^-$  catalysis of unionized benzonatate;  $k_{HPO_4^{2-}}$ , second-order rate constant for  $HPO_4^{2-}$  catalysis of unionized benzonatate;  $k_{HCO_3^-}$ , second-order rate constant for  $HCO_3^-$  catalysis of unionized benzonatate;  $k_{CO_3^{2-}}$ , second-order rate constant for  $CO_3^{2-}$  catalysis of unionized benzonatate;  $k_{OH}$ , second-order rate constant for specific base-catalysis; and  $K_a$ , dissociation constant of benzonatate.

The values of above ten rate constants and one dissociation constant were obtained from non-linear curve fitting of the experimental  $k_{obs}$  values and the corresponding concentrations of  $[H^+]$ ,  $[OH^-]$ ,  $[HAc]$ ,  $[Ac^-]$ ,  $[H_2PO_4^-]$ ,  $[HPO_4^{2-}]$ ,  $[HCO_3^-]$ ,  $[CO_3^{2-}]$  by using Eq. (6). The following related parameters were obtained, where

$$k_H^+ = 7.68 \times 10^{-4} \text{ M min}^{-1};$$

$$k_{OH} = 1.73 \text{ M min}^{-1}; K_a = 2.34 \times 10^{-5} \text{ M}$$

$$k_{HAc}^+ = 5.54 \times 10^{-5} \text{ M min}^{-1};$$

$$k_{HAc} = 7.98 \times 10^{-6} \text{ M min}^{-1}$$

$$k_{Ac^-}^+ = 2.01 \times 10^{-5} \text{ M min}^{-1}$$

$$k_{Ac^-} = 2.20 \times 10^{-5} \text{ M min}^{-1}$$

$$k_{H_2PO_4^-} = 5.26 \times 10^{-4} \text{ M min}^{-1};$$

$$k_{HPO_4^{2-}} = 1.98 \times 10^{-12} \text{ M min}^{-1}$$

$$k_{HCO_3^-} = 4.27 \times 10^{-11} \text{ M min}^{-1};$$

$$k_{CO_3^{2-}} = 8.16 \times 10^{-12} \text{ M min}^{-1}$$

The logarithmic values of apparent hydrolysis rate constants ( $k_{obs}$ ) were plotted as a function of pH in Fig. 3. The close circle points corresponded to the experimental data and the curve plotted in Fig. 3 was according to Eq. (6). The well-fitted by Eq. (6) was observed. In Eq. (6), the apparent hydrolysis rate constants of benzonatate were

given as the sum of five processes: (1) specific acid-catalysis; (2) acetate buffer catalysis; (3) phosphate buffer catalysis; (4) carbonate buffer catalysis; and (5) specific base-catalysis. The acid-catalyzed rate constant was significantly less than the base-catalyzed rate constant by about 2000-fold. Catalysis of benzonatate by buffers was observed in the pH range of 3.61–11.50 and the related catalytic rate constants were compatible to the acid-catalyzed rate constant. From Eq. (6), the fitted dissociation constant,  $K_a$ , was  $2.34 \times 10^{-5}$ , which corresponded to a  $pK_a$  of 4.63. This value was very close to the value of 4.91 which was determined from titration method.

In conclusion, a HPLC method was developed to completely separate benzonatate and its hydrolysis product. The prominent base-catalyzed breakdown of the ester bond and slower acid-catalyzed hydrolysis suggested that benzonatate should be prepared in the solutions with pH 3–7 to maximize its stability.

## Acknowledgements

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