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The Inherent Reactor Kinetics for Transformation of Geniposidic Acid from Geniposide in a Microreactor

Chiu-Lan Hsieh[1], Wang-Chi Hsieh[2], Ping-Xiao Lin[2], Robert Y. Peng[3,4*]

1Graduate Institute of Biotechnology, Changhua University of Education, 1 Jin-De Road, Changhua, Taiwan 50007

2Day Spring Biotech Co., Ltd., 6F., No.128, Section 2, Chong-De Rd., Bei-Tun District, Taichung City 40653, Taiwan.

3Research Institute of Biotechnology, Hungkuang University, 34 Chung-Chie Road, Shalu County, Taichung Hsien, Taiwan 43302

4Research Institute of Medical Sciences, Taipei Medical University, 250 Wu-Xin St., Taipei 100

ABSTRACT

The ripe fruits of *Gardenia jasminoides* Ellis (Rubiaceae) (GJ) are widely used in chemical, food and medicinal industries. Crocin and geniposide, the main constituents of GJ, have shown a diversity of biological activities including sedative, anti-inflammatory and antipyretic. We propose some new bioactive chemicals could be derived from geniposide. The optimum transformation condition of geniposide into geniposidic acid still remains unclear. In order to develop a reactor, the information about the inherent reaction kinetics is required. In a microreactor (V =62.8 mL), geniposide (0.01 mole/L, 20 mL) and NaOH (0.1 equivalent/L, pH=13, 10mL) were left to react at 80, 70, 60, 50, and 40 ^{o}C and tracked with HPLC. Results indicated that the reaction obeyed the pseudo-first order kinetics, the corresponding pseudo-first order rate constants (k_1') were 11.064 h^{-1}, 8.682 h^{-1}, 2.400 h^{-1}, 1.021 h^{-1}, and 0.750 h^{-1}, and the fractional conversions were 73.4%, 60.5%, 38.6%, 43.6%, and 51.8% at 0.50, 0.50, 0.833, 1.00, and 2.00 h. The energy of activation was 8.751 kJ mol^{-1}. Conclusively, this transformation obeys the pseudo-first order kinetics with a low energy of activation, 8.751 kJ mol^{-1}. The optimum transformations at 80^{o}C and 70^{o}C for 0.5 h were 73.4% and 60.5%, respectively.

Keywords: Fructus Gardeniae, geniposide, geniposidic acid, inherent reaction kinetics, microreactor.

I. INTRODUCTION

Fructus Gardeniae is the desiccated ripe fruits of *Gardenia jasminoides* Ellis (Rubiaceae). This plant grows widely in the southern China including mostly the south part to the Yantze River. The harvest seasons are Autumn and Winter. It can be administered either fresh, scotched or baked. In Traditional Chinese Medicine it has shown biological activities covering cholagogue, sedative, diuretic, anti-inflammatory and antipyretic effects (Ni et al., 2006) and usually it is used in a compound prescription. As cited, the medicinal constituents of which mainly involve gardenoside (Figure 1) (Paik et al., 2001) geniposide (an iridoid glycoside) (GPS),

and genipinic acid (GNPA) (Lou and Qin, 1995; Zhang, 1993; Yen, 1994; Hsu, 1996; Ting and Huang, 1998; Yeh and Zhang, 1999). Recently, Wen et al. indicated the presence of these three principles in the Fructus Gardeniae and the occurrence of GPS was found to range within 4.12.1-7.13% (Wen et al., 2011).

In food industries, its primary uses are attributed to the yellow coloring chemicals like crocin (Hendry and Houghton, 1996), blue color derivative like Gardenia Blues (Paik et al., 2001) (Figure 1), and red color derivative like Gardenia Red (Mori et al., 1999; Dong, 2007; Lu et al., 2008) (Figure 1).

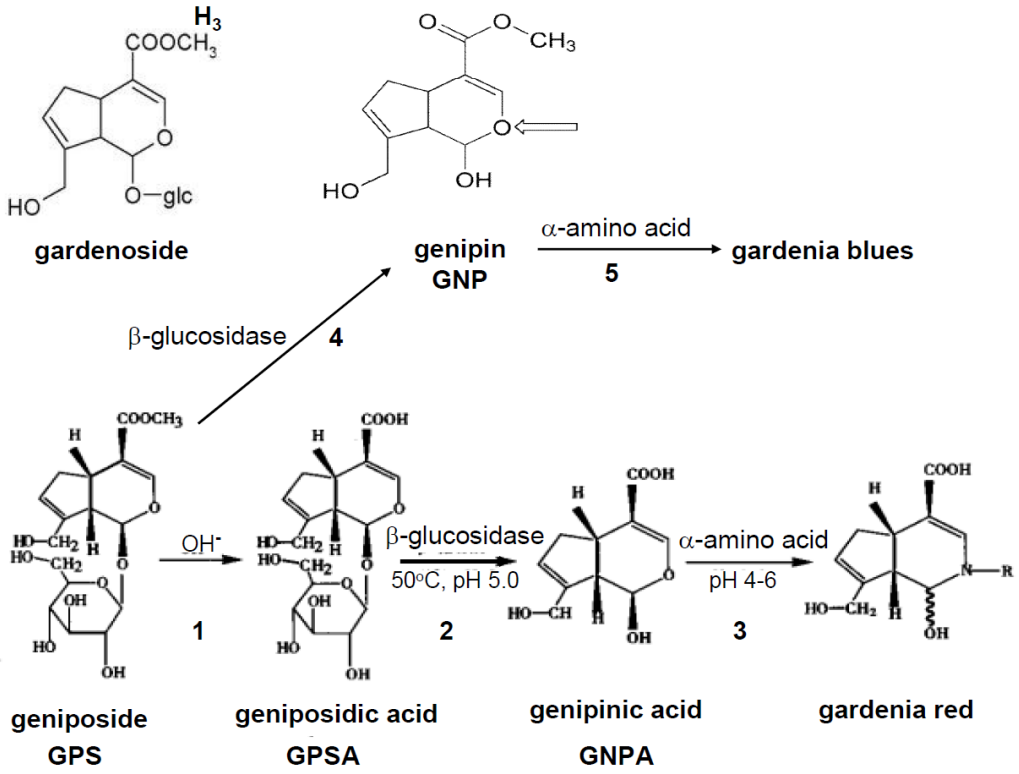


Figure 1. Struture of geniposide, geniposidic acid, genipinic acid and genipin. The alkaline hydrolysis of geniposide (GPS) (reaction 1) yields geniposidic acid (GPSA) that can be deglucosylated (reaction 2) by β-glucosidase to produce genipinic acid (GNPA) and then Gardenia Red if coupled with some selected α-amino acids (reaction 3) (modified from Dong, 2007; Lu et al., 2008). The reaction path from geniposide to genipin (GNP) by action of β-glucosidase (reaction 4) and then to Gardenia Blues (reaction 5) is modified from Pike et al., 2001.

Mori et al. produced the Gardenia Red starting from geniposide. The first conversion step of which involved the alkaline hydrolysis of geniposide (Mori et al., 2007). The production of Gardenia Red also has been demonstrated by Xie et al. (2011) in which geniposidic acid (GPSA) was first deglucosidylated using the enzyme mixture (the main enzyme β-glucosidase with activity unit of 45.2 Ug^{-1} was obtained from *Aspergillus niger*) at pH 4.5 and a temperature 40°C to form GNPA, the latter then

coupled with different kinds of amino acids to produce Gardenia Reds (Xie et al., 2011) (Figure 1). The reaction condition used was different from that of Dong (2007). In the latter, the alkaline demethylaion and the deglucosidylation of GPS was completed in a single step using NaOH at pH 12 and 80^{o}C (Dong, 2007), while the hydrolysis with β-glucosidase was applied at 50^{o}C and pH 5.0 for a reaction period of 48 h (Dong, 2007).

A diversity of medicinal effects have been cited for geniposide acting as hepatic-protective (Peng et al., 2003), antidiabetics (Xie and Jin, 2008) and antithrombotic bioactivity (Suzuki et al., 2001).

The active form of geniposide is genipin (GNP) (Figure 1), a deglucosidylated product of geniposide. Animal study has revealed that many bioactivities are associated with GNP in a dose dependent manner. As the dose of pure GNP can be precisely controlled, its clinical curative effects can be improved without triggering its toxicity (Wang et al., 2009; Cao et al., 2010; Lelono et al., 2009). In addition to the biological effects already mentioned, GNP also exhibits neurotoxicity inhibitory, (Yamazaki et al., 2009) and antidepressant effects (Tian et al., 2010).

The naturally occurring GNP is rare, and more importantly, GNP is quite unstable during isolation and storage (Lu et al., 2008). Hence generally, GNP is prepared by enzymatic hydrolysis of GPS using β-glucosidase (Figure 1) (Lu et al., 2008). Apparently, the low productivity and high production cost has limited the application of GNP (Lu et al., 2008).

Considering the annual vast harvest of Fructus Gardeniae around our local area and the amazing therapeutic effects of GNP, we predict that a diversity of bioactive compounds possibly could be derived from GPS. In this present study we explored the process optimization for the production of GPSA adopting the alkaline hydrolysis (reaction 1 in Figure 1).

II. MATERIALS AND METHODS

2.1. Chemicals

Authenic geniposide was purchased from Cheng-Du ConBon Bio-Tech CO., LTD (Su-Chuan, China). Geniposidic (GPSA) (purity >98%) was a product of Linchuan Zhixin Bio-Technology Co., Ltd., China).

2.2. Micro-Chemical Reactor

The microreactor used for this investigation was manufactured by the Taichung Fine Machinery Co., Ltd. (Taichung City, Taiwan). A specification of this microreactor is indicated in the figure 2. This micro-chemical reactor has an inner diameter of 40 mm; an inner effective height of 50 mm; a total designed interior volume (TDIV) of 62.8 cm^3; an effective working volume of 50.2 cm^3 (= 80% of TDIV); an optimum working volume of 35 – 50.2 cm^3 (= 55.7%- 80% of TDIV) (Figure 2).

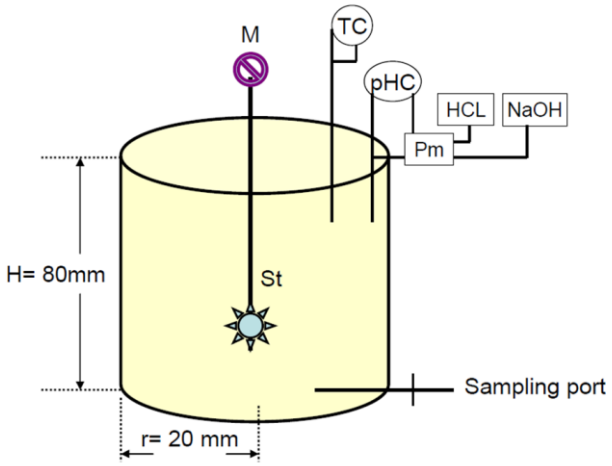


Figure 2. Schematic of micro-chemical reactor used for investigation of inherent reaction engineering.

The specification of this microreactor is: an inner diameter= 40 mm; an inner effective height = 80 mm; total designed interior volume (TDIV) = 62.8 cm^3; an effective working volume of 50.2 cm^3 (= 80% of TDIV); an optimum working volume of 35 cm^3 – 50.2 cm^3 (= 55.7%- 80% of TDIV). M, micromotor. St, stirrer. TC, temperature controller. pHC, pH controller. Pm, micropump

2.3. Preparation of standard solutions

Geniposide (0.20 g) and geniposidic acid (0.184g) were accurately weighed and respectively dissolved in deionized water to make a final volume of 50 mL. The stock solution was successively diluted to 5.000 mM, 2.500 mM, 1.250 mM, 0.625 mM, and 0.3125 mM. An aliquot of 10 μL of the diluted solution was subjected to HPLC analysis to establish the standard curve. The area under each peak was integrated to obtain the concentration.

2.4. Microreactor to access the intrinsic reaction kinetics

As a diversity of factors could affect the reaction rate, i.e. (equation 1)

$$Rate = f(R_m, R_h, R_{ik}) \quad \text{.....1}$$

Here the 'Rate' is the overall reaction rate. R_m denotes the effect of mass transfer, R_h is the effect of heat transfer, and R_{ik} is the effect of inherent reaction kinetics. In order to avoid the interference of R_m and R_h on the inherent kinetic behavior R_{ik}, we performed this study using a microreactor. Since the mass- and heat-transfer would occur instantaneously, the transfer time can be greatly reduced if a microreactor is used instead of a regular larger reactors, like 500 L or a reactor volume > 1000 L. In this experiment, we used a microreactor having a working volume of 50.2 cm^3 (= 80% of TDIV) (Figure 2), so that the intrinsic kinetic behavior can be directly accessed. Hence, equation 1 is reduced to equation 2:

$$Rate = f(R_{ik}) \quad \text{.....2}$$

2.5. Tracking the alkaline hydrolysis of geniposide with HPLC

Briefly, 0.2 g of geniposide (MW = 388.37) was dissolved in deionized water to make a final volume of 50 mL (= 0.01 mole/L). To 20 mL of the geniposide solution, 10 mL of NaOH (0.1 equivalent/L) was added. The mixture was thoroughly agitated and left to react respectively at 80, 70, 60, 50, and 40 ^oC. The sampling intervals were 0, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 40 min, 50 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, 270 min, 300 min, 330 min, 360 min, 390 min, and so on up to 720 min when needed. Each time 1 mL of aliquot was transferred into the Eppendorf tube, 6 μL of 6N HCl was immediately added to suppress the pH value to 2.5 in order to terminate the reaction as soon as possible. When the reaction ceased (This takes about 0.5 min.), 10 μL of the sample aliquot was subjected to HPLC analysis.

2.6. Kinetic analysis

For a second order reaction like this present case (equation 3),

A + B $\rightarrow$ 3

Where A represents genipoxide, B is NaOH and X is the product geniposidic acid.

The rate equation in differential form is

$$dx/dt = k_2(a-x)(b-x) \quad \text{.....4}$$

Here x denotes the concentration of product geniposidic acid, a is the concentration of geniposide,

b is the concentration of [OH^-]. k_2 is the rate coefficient of the second order reaction (equation 4).

Integration of equation 4 leads to

$$\int_0^x dx/(a-x)(b-x)$$
$$=[1/(b-a)]\int_0^x [1/(a-x)]-[1/(b-x)]dx \ldots\ldots$$
$$=k_2\int_0^t dt$$

……..5

Integration and rearrangement of equation 5 yields equation 6:

$$k_2 = 1/t(a-b)\ell n[b(a-x)]/[a(b-x)]$$
$$=2.303/[t(a-b)]\ell og[b(a-x)]/[a(b-x)] \ldots$$

……..6

In this type of alkaline catalyzed hydrolytic reaction, NaOH in reality plays as a catalyst, the concentration of which can be assumed to be constantly unchanged during the whole course of reaction, consequently equation 4 can be simplified to equation 7:

$$dx/dt = k_2(a-x)(OH^-) \ldots\ldots\ldots\ldots\ldots 7$$

or

$$dx/dt = k_2(a-x)(K) \ldots\ldots\ldots\ldots\ldots\ldots 8$$
$$dx/dt = k_1'(a-x) \ldots\ldots\ldots\ldots\ldots\ldots 9$$

Equation 9 actually is a pseudo-first order kinetic equation.

Where

$$k_1' = k_2 * K \ldots\ldots\ldots\ldots\ldots\ldots 10$$

And k_1' is termed 'the pseudo-first order rate constant'.

Integration of Eq. 9 leads to equation 11:

$$\int_0^x dx/[k_1'(a-x)] = \int_o^t dt \ldots\ldots\ldots\ldots 11$$

$$\therefore -[\ell n(a-x)]_0^x = k_1'[t]_0^t \ldots\ldots\ldots\ldots 12$$

Or

$$\ell n[a/(a-x)] = k_1't \ldots\ldots\ldots\ldots 13$$

2.7. HPLC analysis

Hitachi HPLC (Hitachi High Tech, Tokyo, Japan) equipped with an ultraviolet detector L-2400 (Hitachi High Tech) and an L-2130 HTA pump was used for tracking the kinetics of hydrolysis for transformation of geniposide into geniposidic acid in alkaline NaOH environment. The separation column (RP-18/RP-8 DNPH cartridge, ℓ×i.d. =150×4.6 mm) packed with Mightysil RP-18 GP Aqua 250-4.6 (5 μm) (Kanto Chemical Co. Inc. Tokyo, Japan) was used for HPLC analysis. The mobile phase was a mixture of 0.1% H_3PO_4 and 25% methanol which was operated at a flow rate 1.5 mL/min. The detector monitored the effluents at UV 240 nm. The amount of the reactant geniposide and the product geniposidic acid were calculated from the calibration curve established with the authentic chemicals (Xie et al., 2011).

2.8. Arrhenius equation to estimate the activation energy

The van't Hoff and Arrhenius equation takes the form (equation 14)

$$k = Ae^{-E/RT} \ldots\ldots\ldots\ldots 14$$

Where k is the rate constant, E is the activation energy, R is the gas constant, T is the related Kelvin temperature, and A is the proportionality constant that involves the number of collision and the probability function of the collision.

In order to test the Arrhenius equation (equation 14), logarithm was taken at both sides to give equation 15:

$$\ell nk = \ell nA - (E/R)(1/T) \ldots\ldots\ldots\ldots 15$$

If the Arrhenius law applies, a plot of ℓnk against $1/T$ will be a straight line, and the slope (which has the unit of K) will be $-E/R$. Where the values of gas constant R as commonly used is 8.31441 $JK^{-1}mol^{-1}$ (SI unit).

III. RESULTS AND DISCUSSION

3.1. HPLC tracking of the alkaline hydrolysis

Figure 3 is the established calibration curves of geniposide (GPS) and geniposidic acid (GPSA). The retention time of geniposide and geniposidic acid on the HPLC was 14.02-14.91 min and 3.99-4.37 min, respectively (The variation of retention times occurred due to the slight instability of the HPLC column status during the experiment).

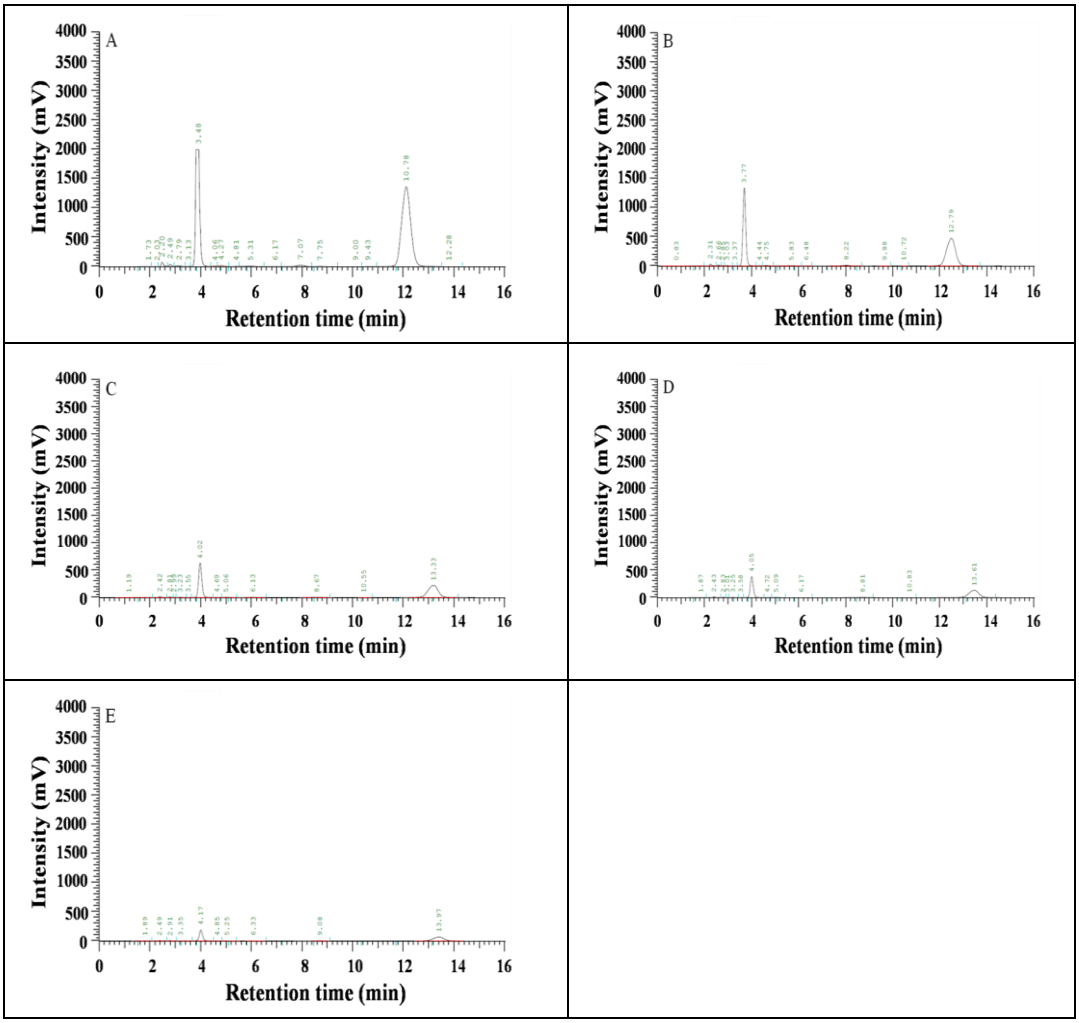


Figure 3a. HPLC of standard geniposide and geniposidic acid samples.

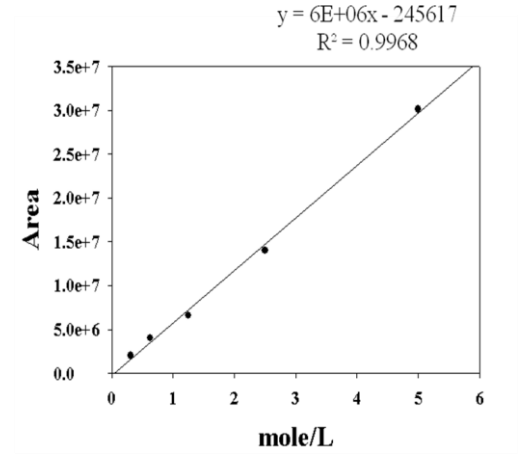


Figure 3b. Standard curve for geniposide (GPS).

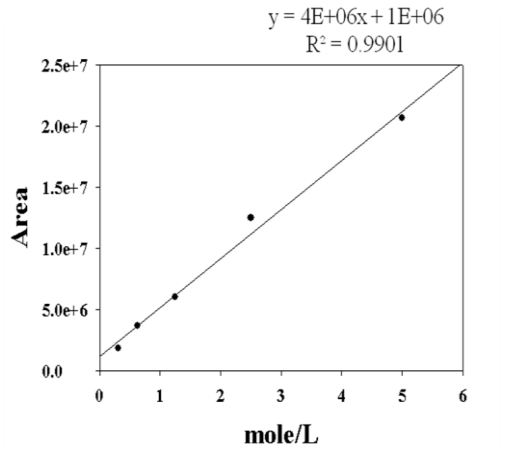
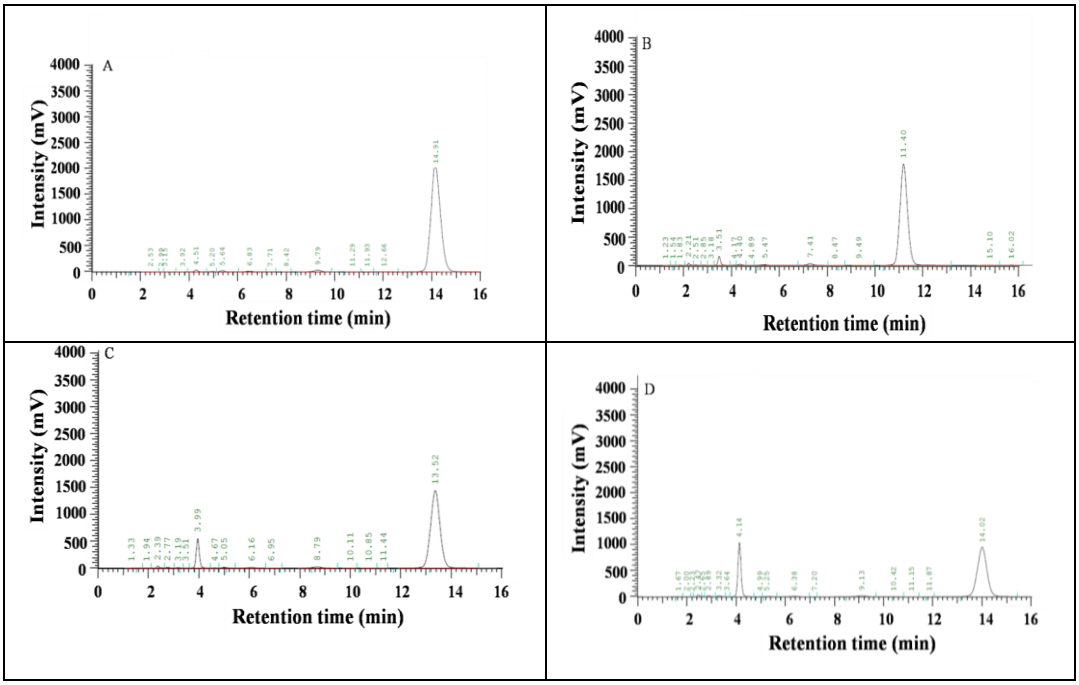


Figure 3c. Standard curve for geniposidic acid (GPSA).

Figure 3. Establishment of the calibration curve for geniposide and geniposidic acid. a) HPLC of standard geniposide and geniposidic acid. A) 5 mole/L. B) 2.5 mole/L. C) 1.25 mole/L. D) 0.625 mole/L, and E) 0.3125 mole/L. b) Standard curve for geniposide (GPS). c) Standard curve for geniposidic acid (GPSA).

The HPLC spectra indicated that both the reactant (geniposide) and the product (geniposidic acid) were rather resistant to dilute alkali (0.01 euivalent/L NaOH = 0.01 mole/L) and heat treatment (Figure 4A-Fig. 4Z). At 80 ^{o}C, the hydrolytic reaction occurred very early at 5 min after exposure (Figure 4B). As can be clearly seen, the conversion proceeded in one mole to one mole pattern without any side reaction (Figure 4A-Figure 4H) and the reaction was completed at 50 min when reaction was conducted at 80 ^{o}C (Figure 4J). The time required for complete hydrolysis became longer when the reaction temperature was reduced (Figure 4A-Figure 4Z).



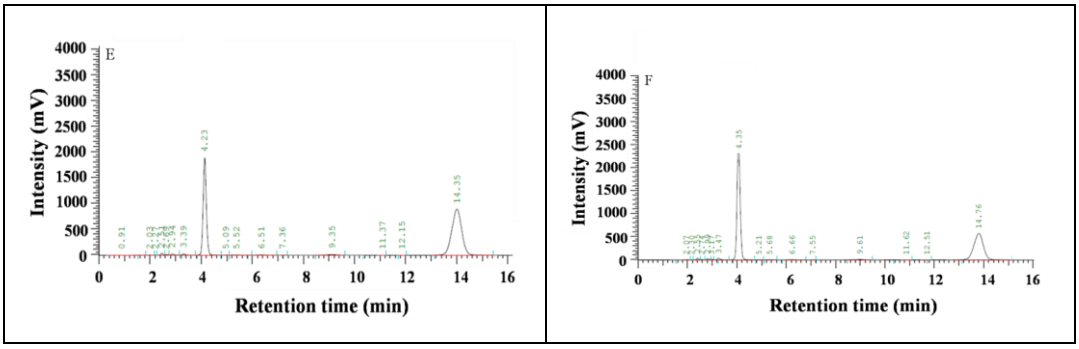


Figure 4a). Alkaline hydrolysis of geniposide at 80°C

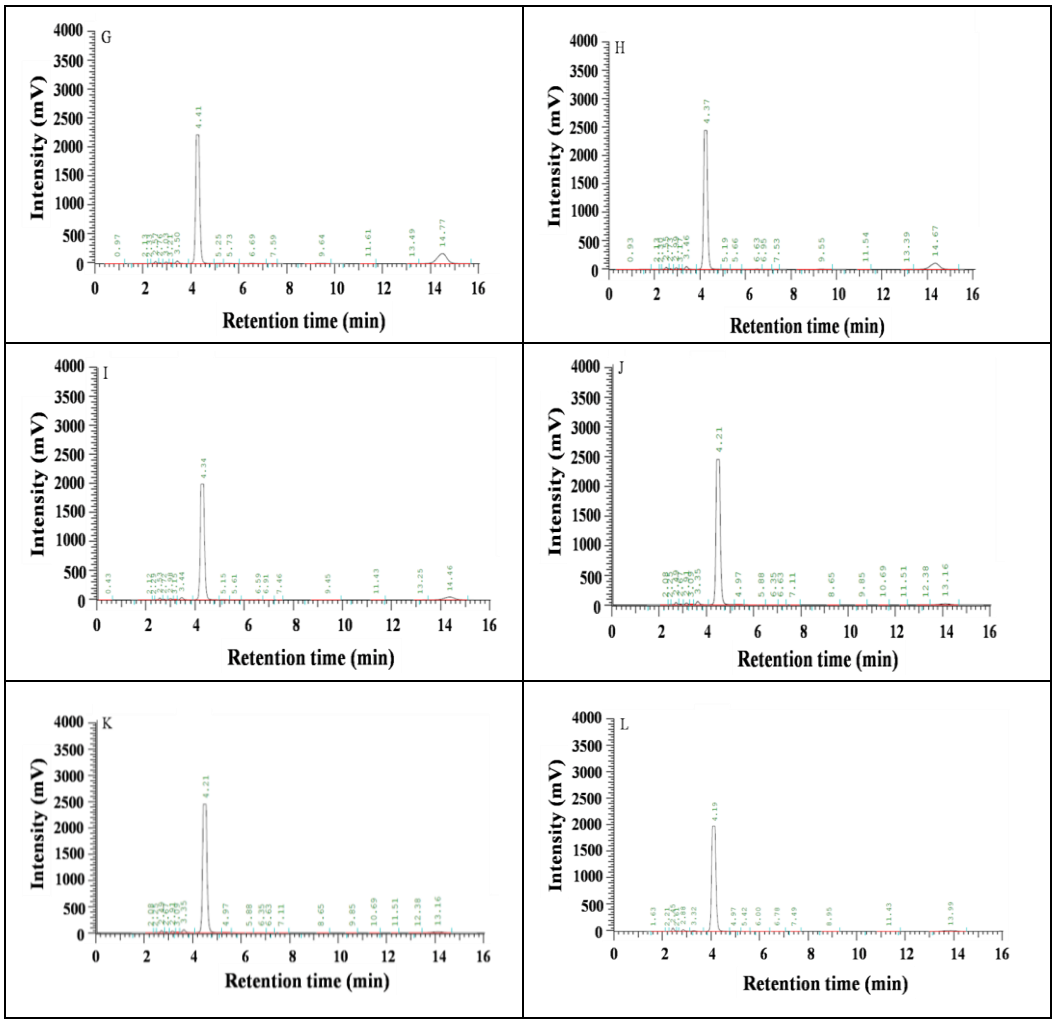


Figure 4a). Alkaline hydrolysis of geniposide at 80°C (continued).

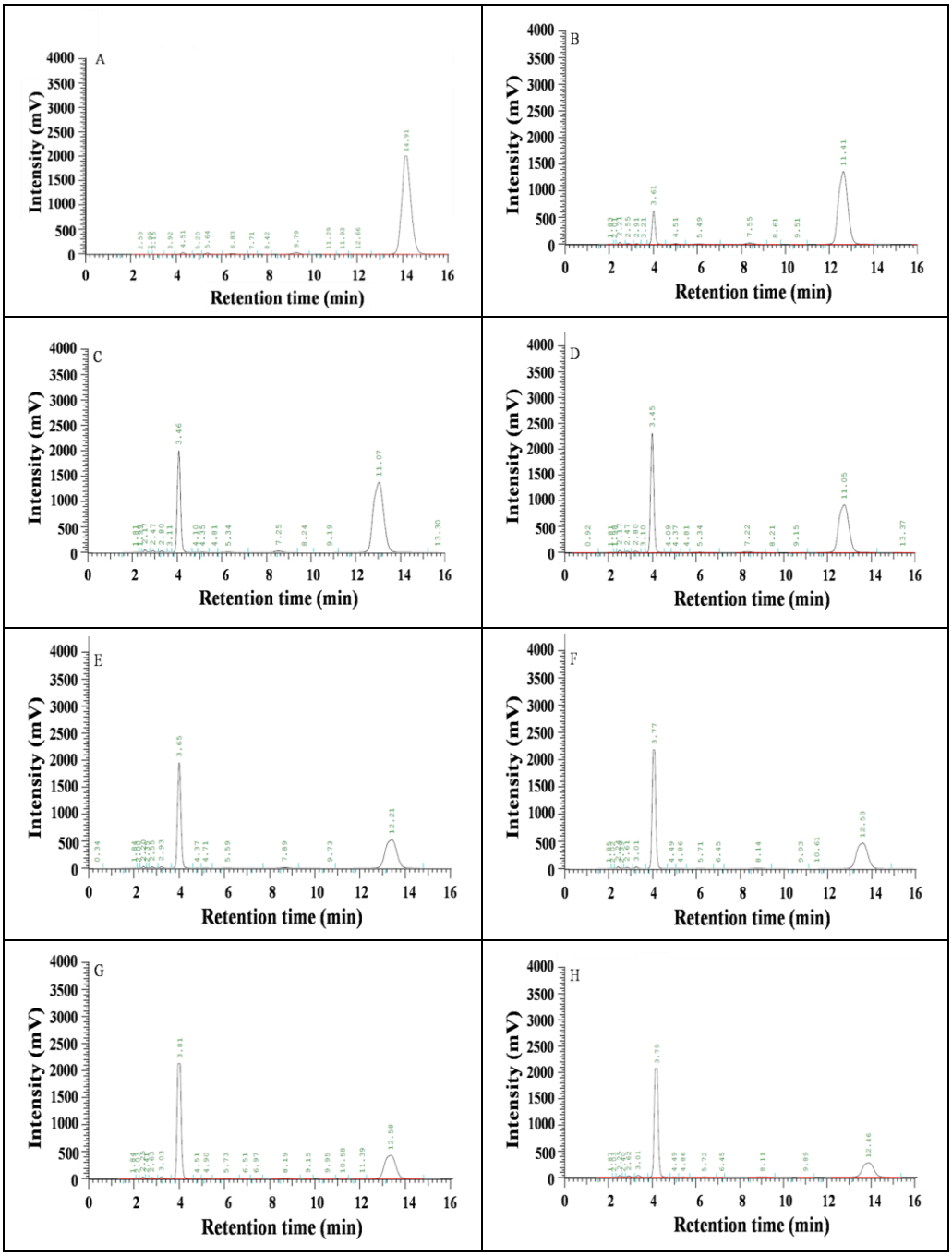


Figure 4b). Alkaline hydrolysis of geniposide at 70°C.

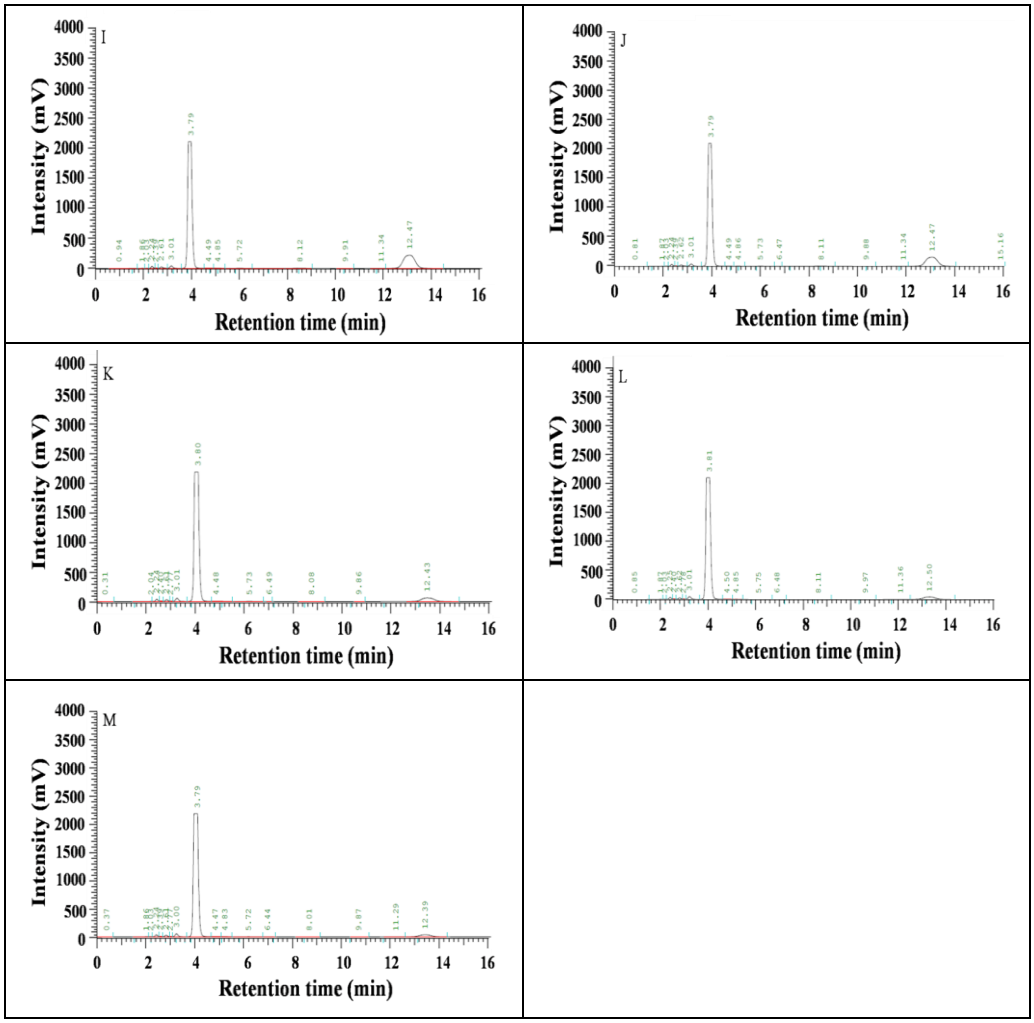
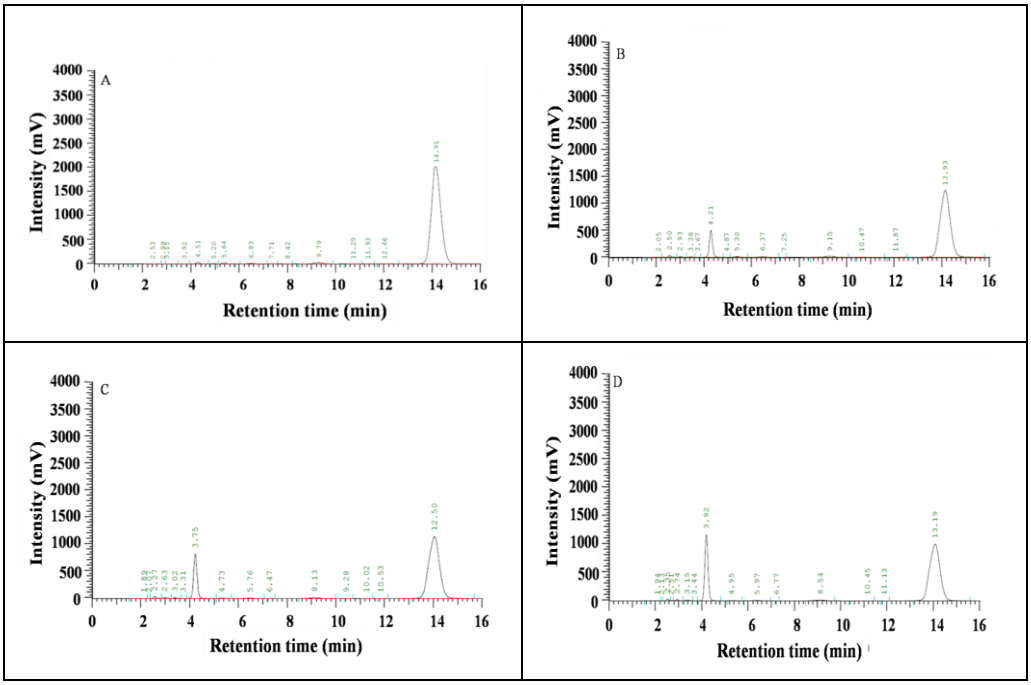


Figure 4b). Alkaline hydrolysis of geniposide at 70°C. (continued).



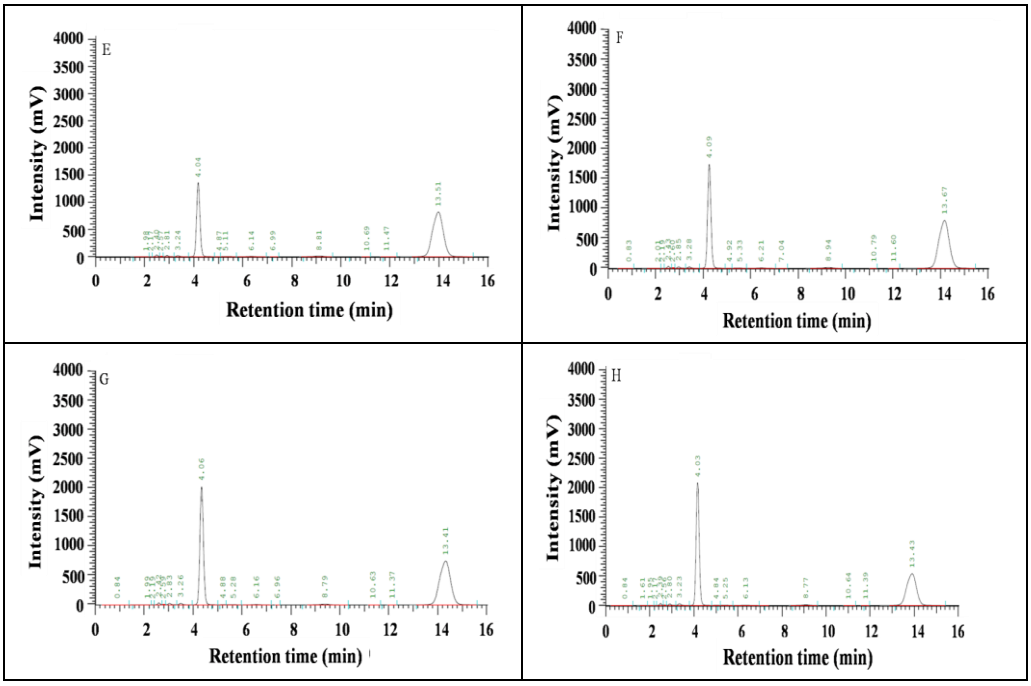
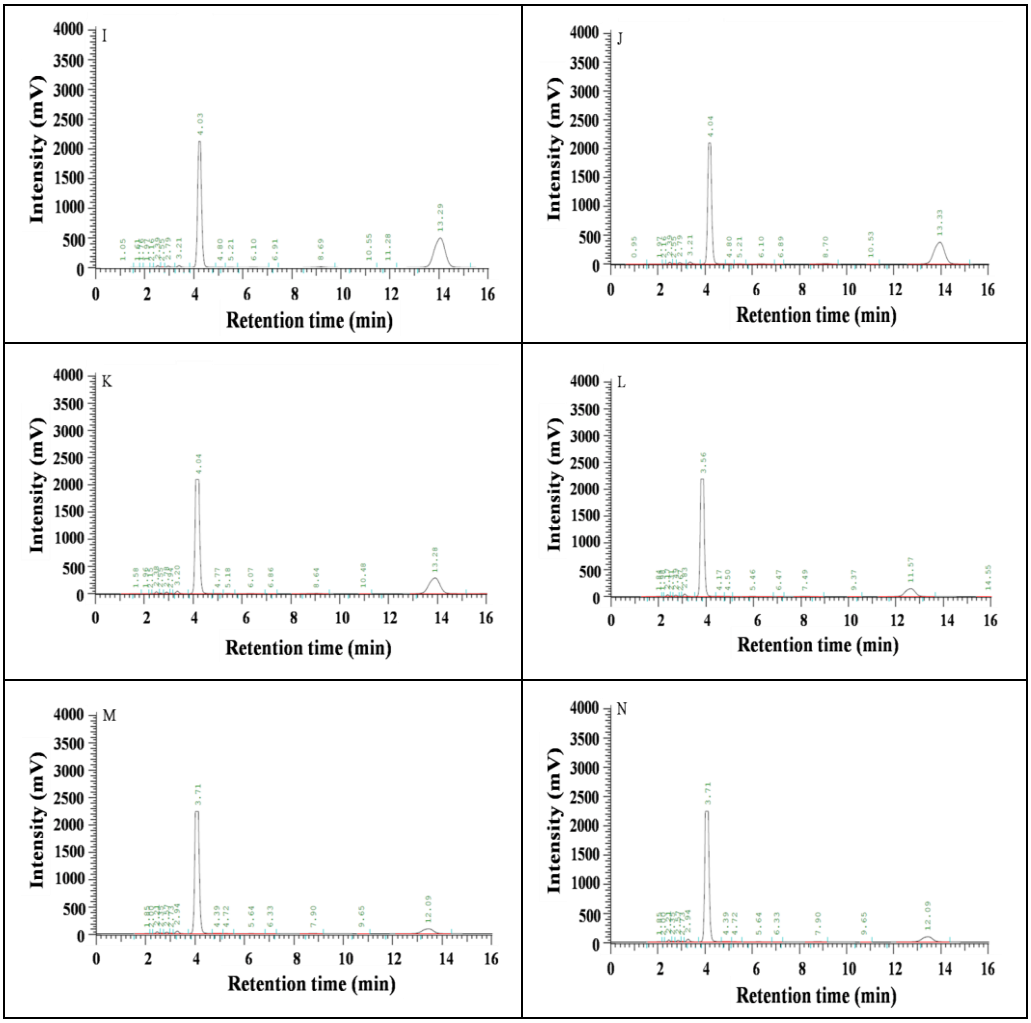


Figure 4c). Alkaline hydrolysis of geniposide at 60°C.



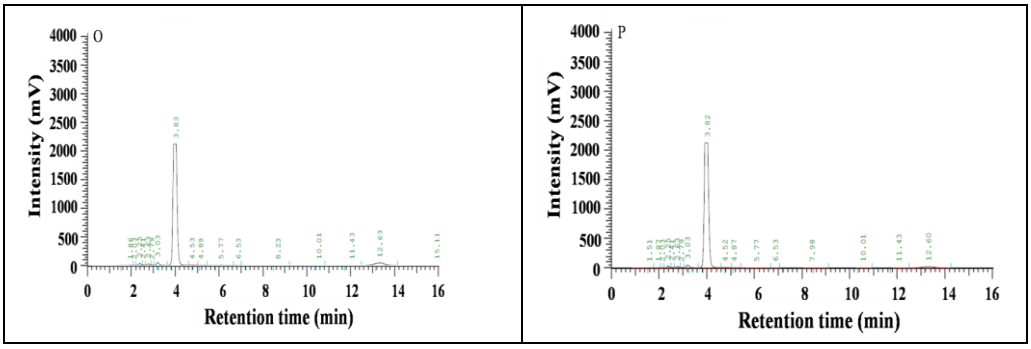


Figure 4c). Alkaline hydrolysis of geniposide at 60°C. (continued).

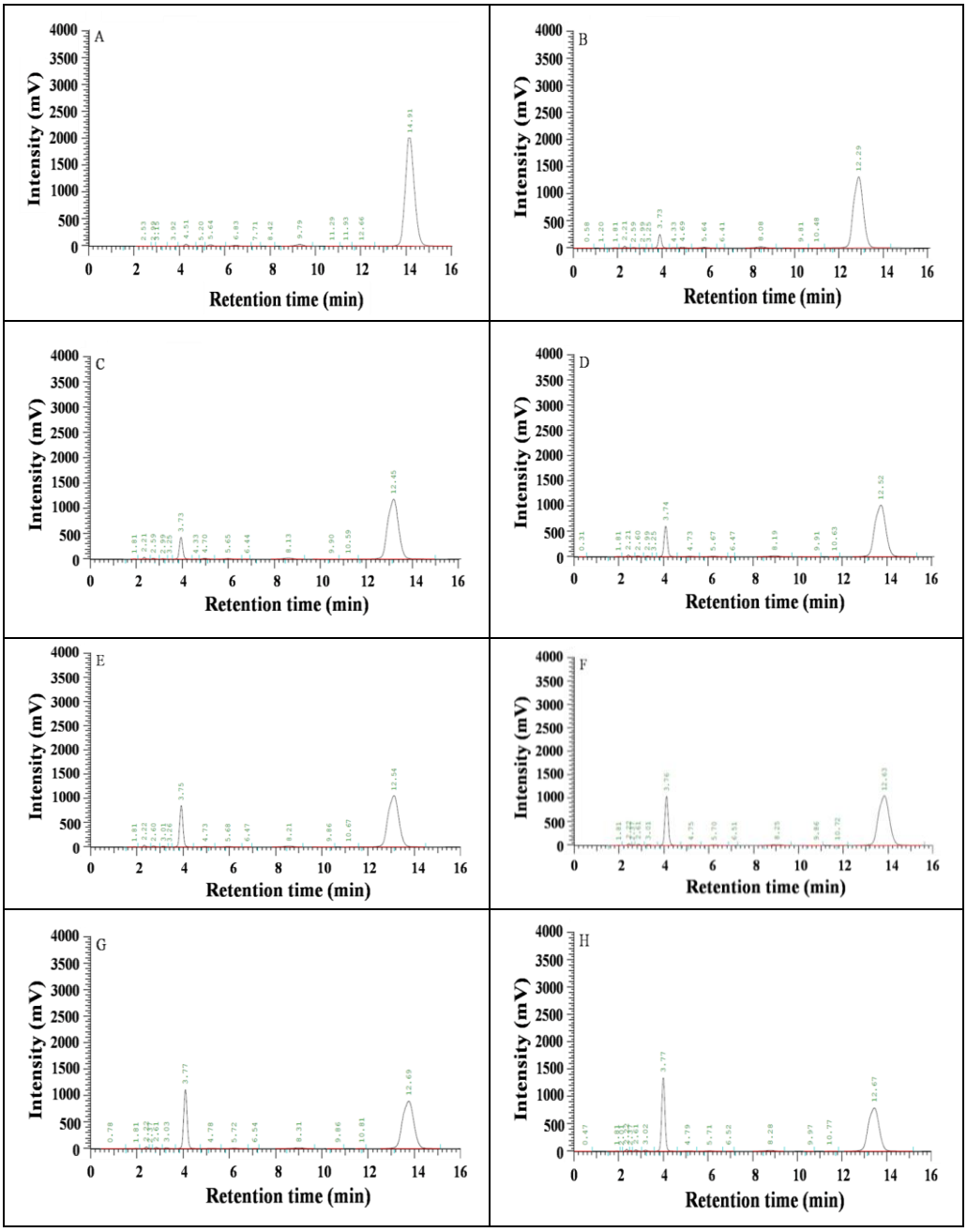


Figure 4d). Alkaline hydrolysis of geniposide at 50°C.

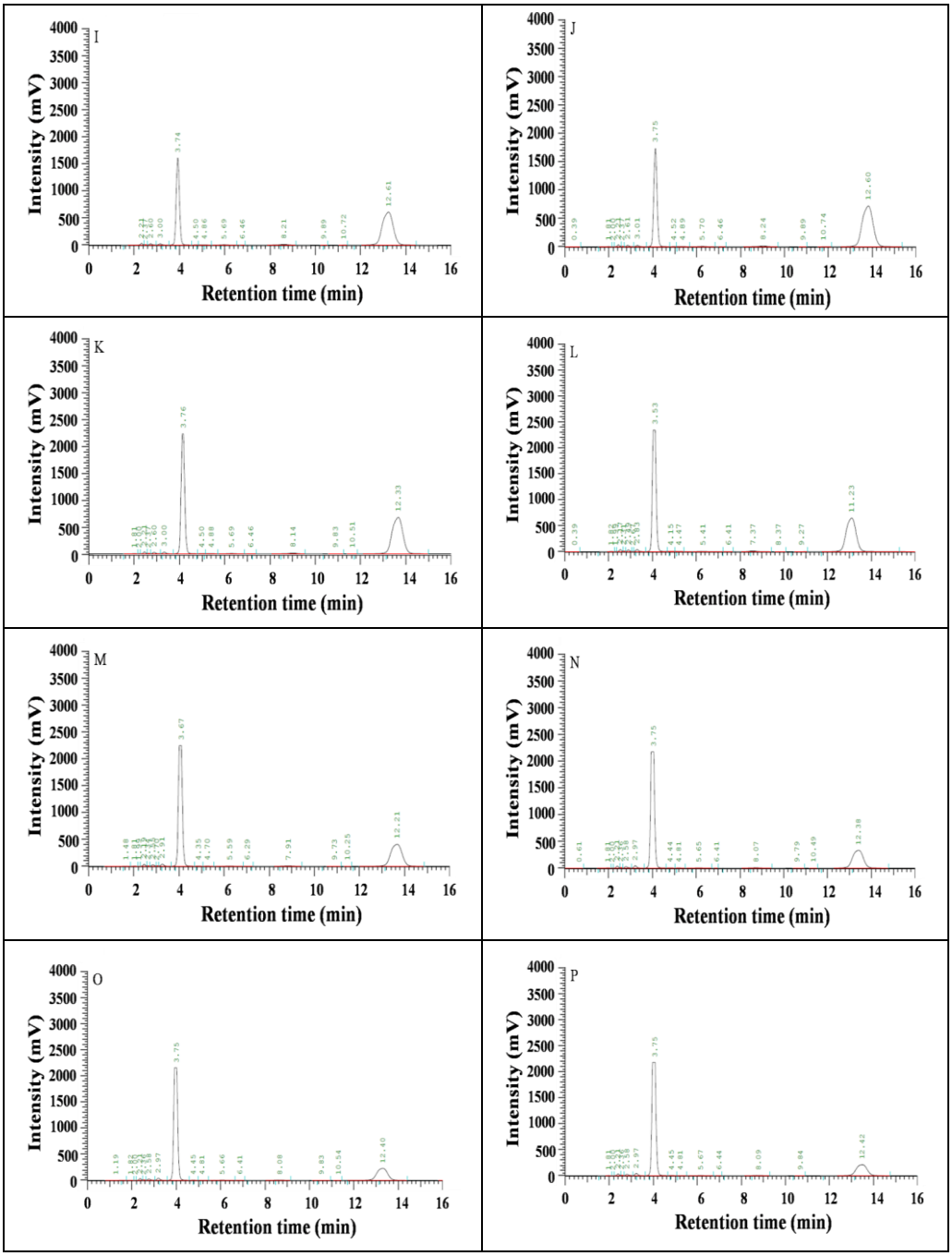


Figure 4d). Alkaline hydrolysis of geniposide at 50°C. (continued).

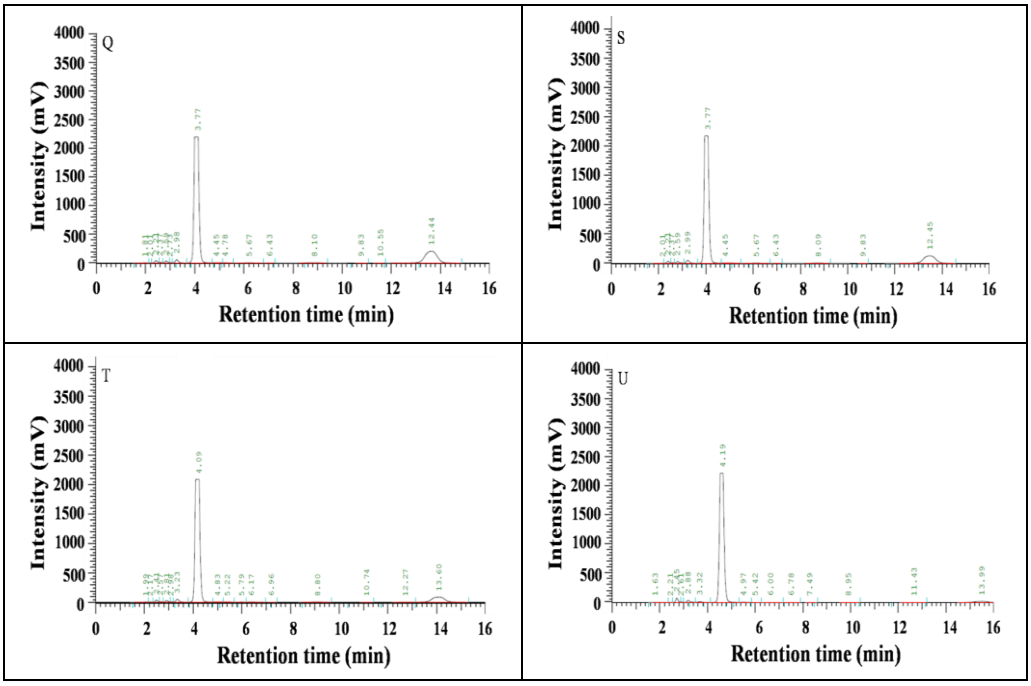
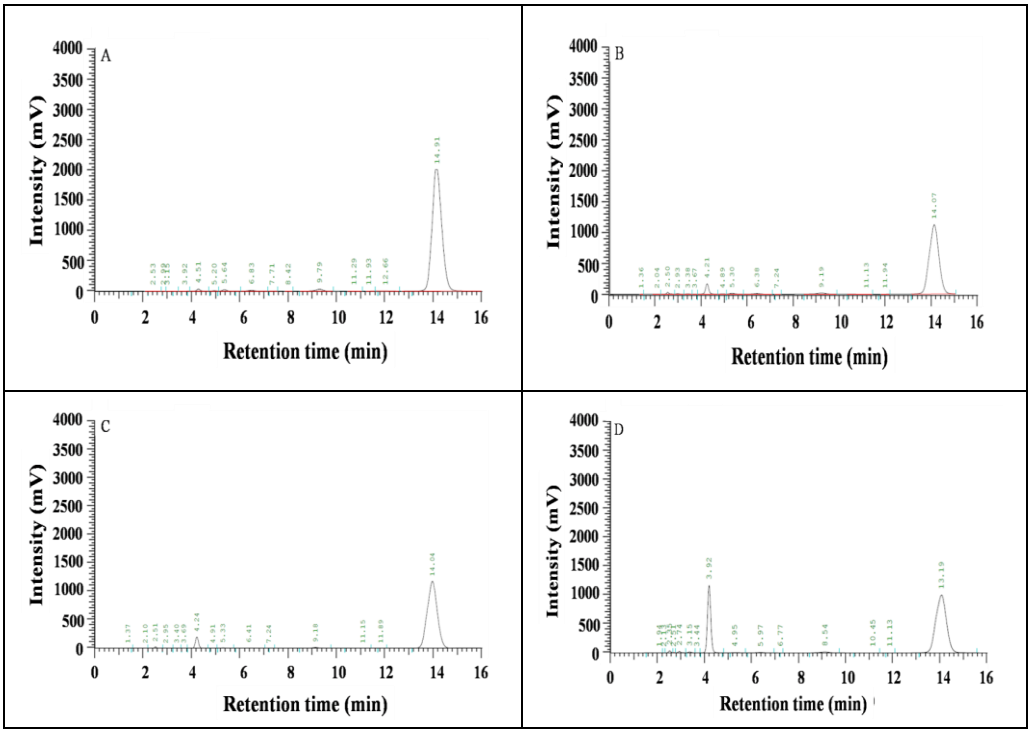


Figure 4d). Alkaline hydrolysis of geniposide at 50°C. (continued).



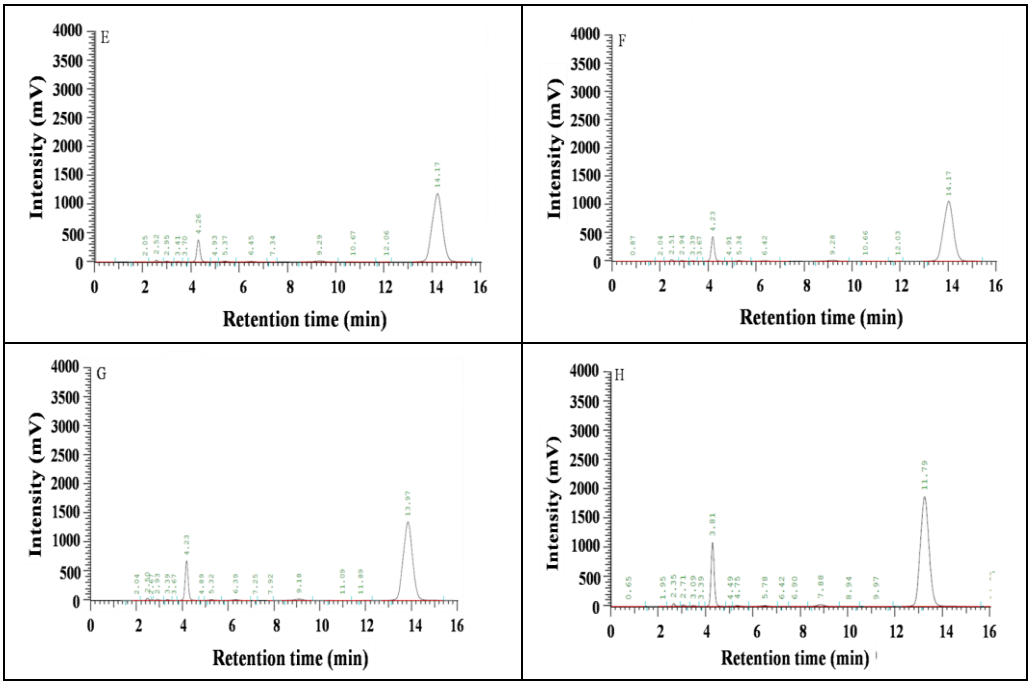
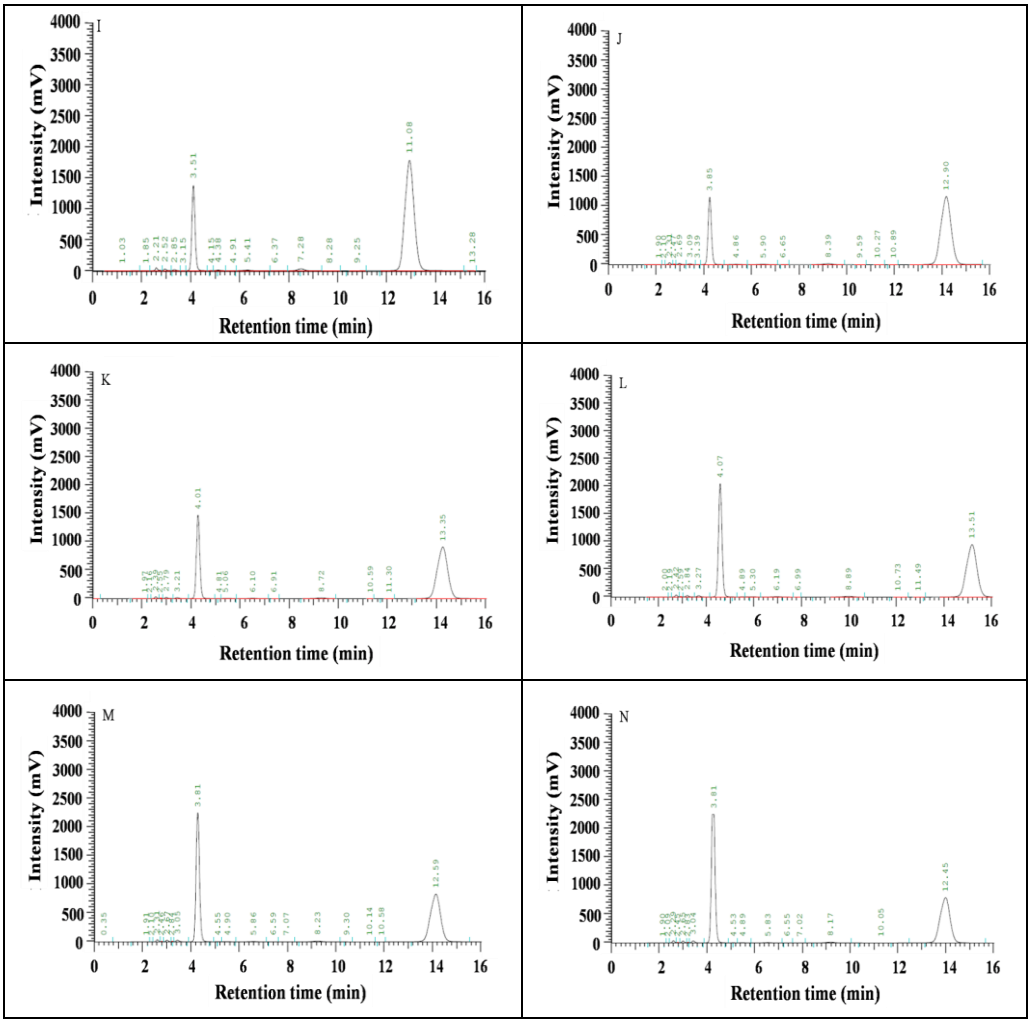


Figure 4e) Alkaline hydrolysis of geniposide at 40°C.



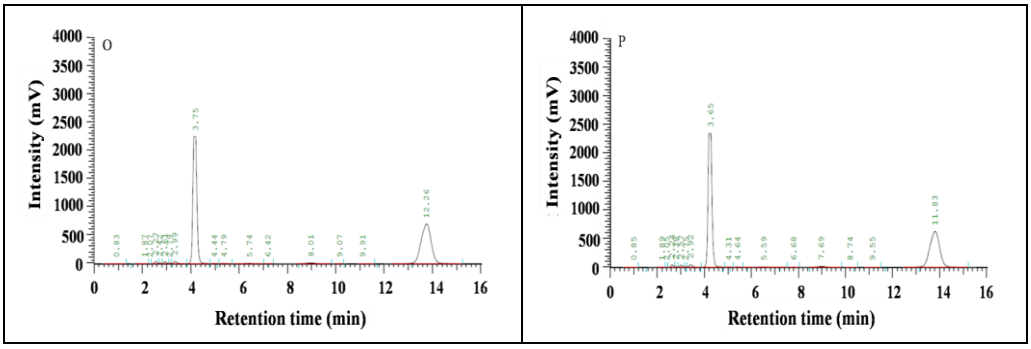


Figure 4e) Alkaline hydrolysis of geniposide at 40°C. (continued).

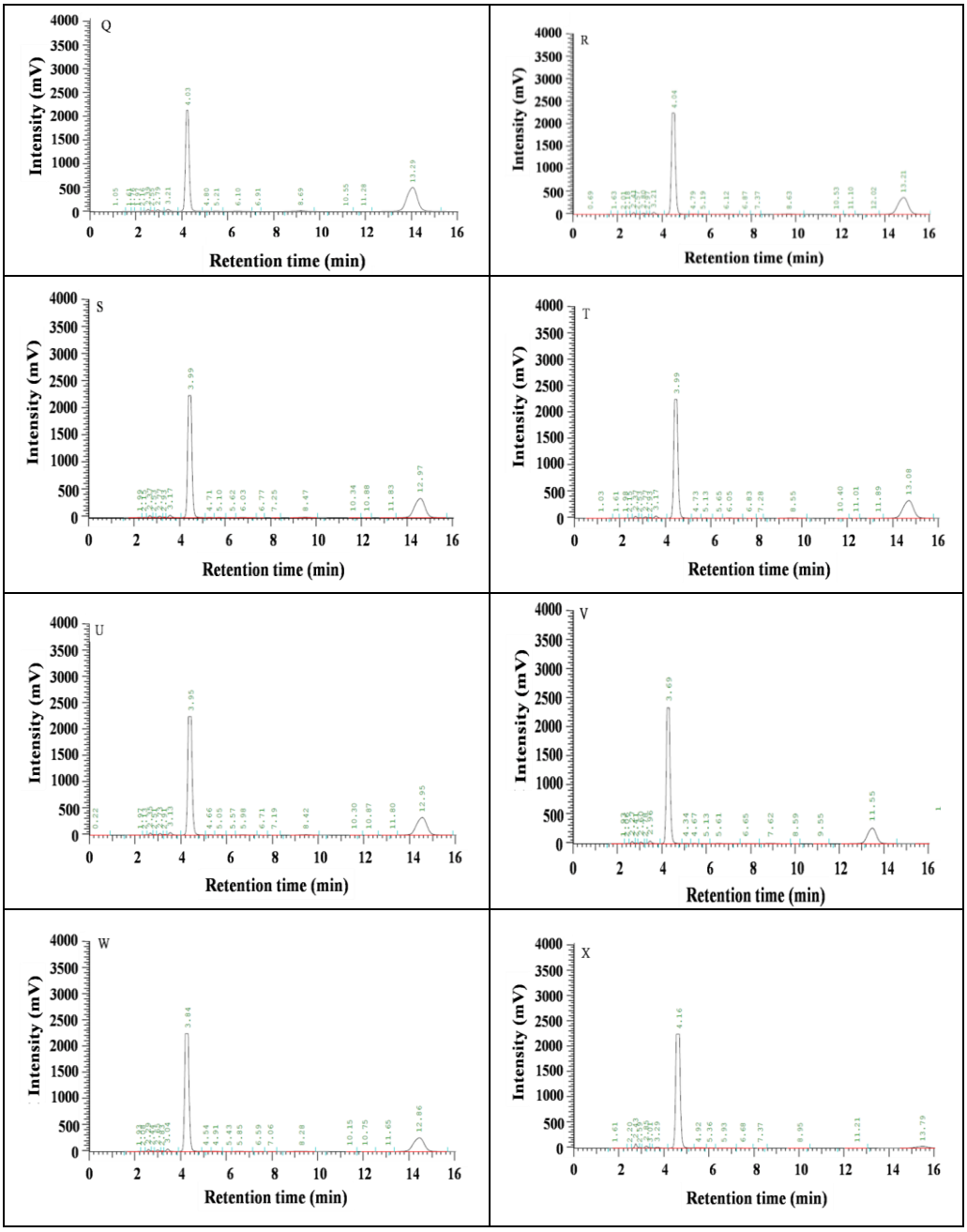


Figure 4e) Alkaline hydrolysis of geniposide at 40°C. (continued).

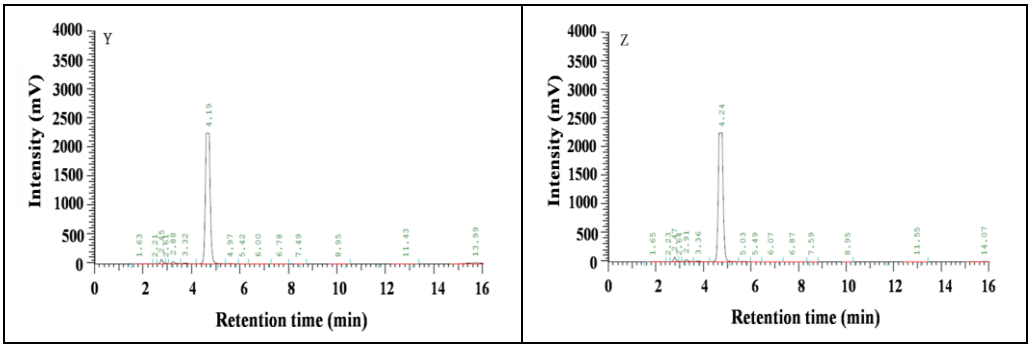


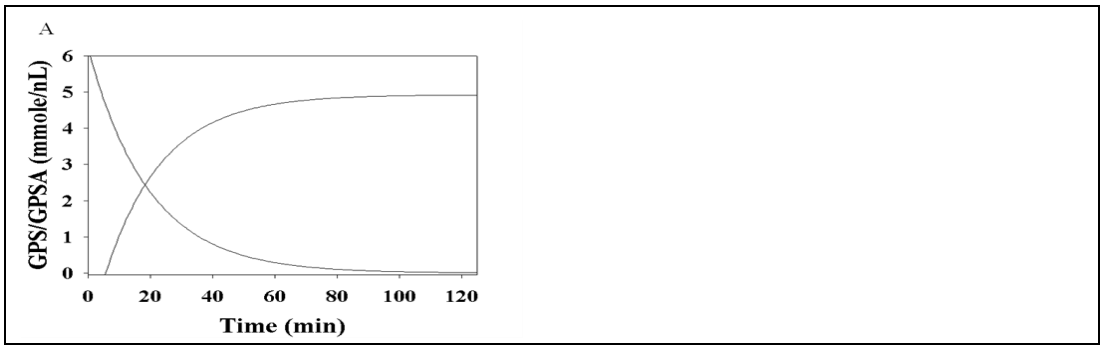
Figure 4e) Alkaline hydrolysis of geniposide at 40°C. (continued).

Figure 4. HPLC tracking for the alkaline hydrolysis for transforming geniposide into geniposidic acid.

a) At 80°C tracked by HPLC analysis vs. reaction time (in min). A) 0, B) 5, C) 10, D) 15 E) 20, F) 25, G) 30, H)40, I) 50, J) 60, K) 90, and L) 120.
b) At 70°C tracked by HPLC analysis vs. reaction time (in min). A) 0, B) 5, C) 10, D) 15 E) 20, F) 25, G) 30, H)40, I) 50, J) 60, K) 90, L) 120, and M) 150.
c) At 60°C tracked by HPLC analysis vs. reaction time (in min). A) 0, B) 5, C) 10, D) 15 E) 20, F) 25, G) 30, H)40, I) 50, J) 60, K) 90, L) 120, M) 150, N) 180, O) 210, and P) 240.
d) At 50°C tracked by HPLC analysis vs. reaction time (in min). A) 0, B) 5, C) 10, D) 15 E) 20, F) 25, G) 30, H)40, I) 50, J) 60, K) 90, L) 120, M) 150, N) 180, O) 210, and P) 240, Q) 270, R) 300, S) 330, T) 360, and U) 390.
e) At 40°C tracked by HPLC analysis vs. reaction time (in min). A) 0, B) 5, C) 10, D) 15 E) 20, F) 25, G) 30, H)40, I) 50, J) 60, K) 90, L) 120, M) 150, N) 180, O) 210, and P) 240, Q) 270, R) 300, S) 330, T) 360, U) 390. V) 420, W) 450, X) 540, Y) 630, and Z) 720.

3.2. Kinetic parameters obtained from the alkaline hydrolysis of geniposide

The reaction parameters for the alkaline hydrolysis of geniposide are listed in Table 1. The initial concentration of geniposide varied from 4.15 to 4.70 mole/L due to the measurement errors (Figure 5, Table 1).



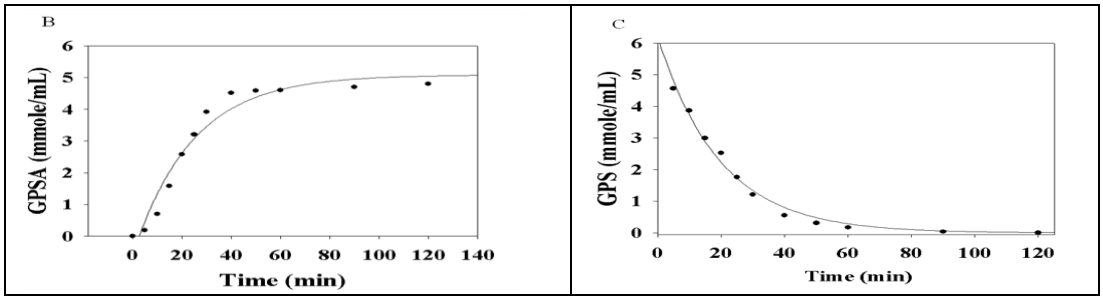


Figure 5a). Hydrolysis of GPS in 0.1M NaOH at 80°C. Reaction time: 2 h. (B) GPSA. (C) GPS.

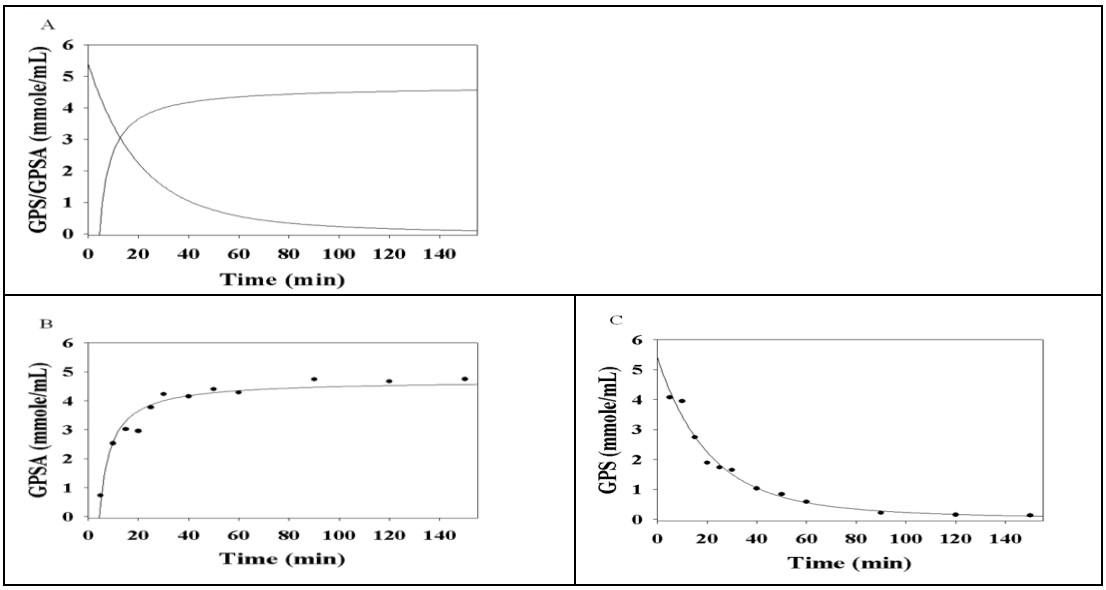


Figure 5b). Hydrolysis of GPS in 0.1M NaOH at 70°C. Reaction time: 2.5 h. (B) GPSA. (C) GPS.

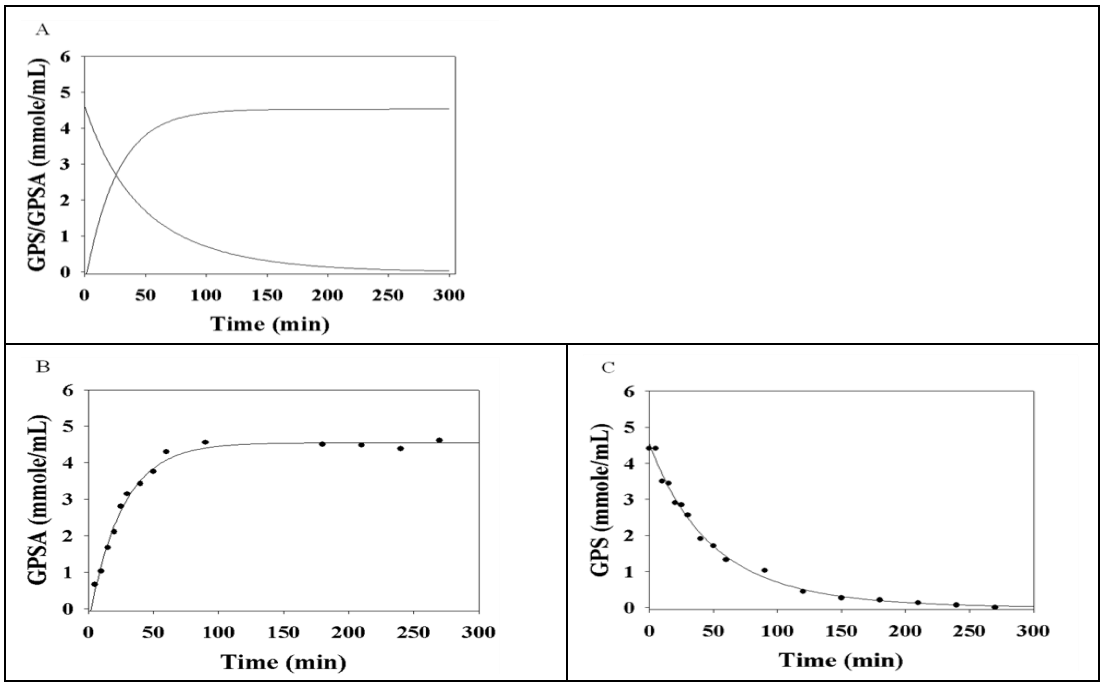


Figure 5c). Hydrolysis of GPS in 0.1M NaOH at 60°C. Reaction time: 5 h. (B) GPSA. (C) GPS.

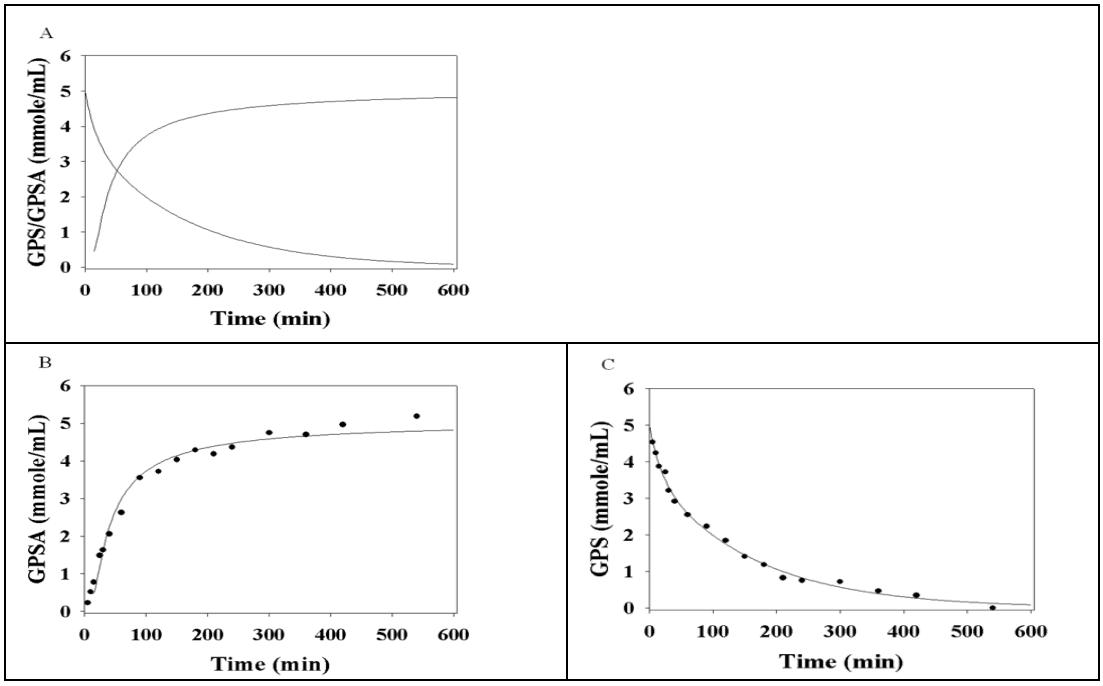


Figure 5d). Hydrolysis of GPS in 0.1M NaOH at 50°C. Reaction time: 9 h. (B) GPSA. (C) GPS.

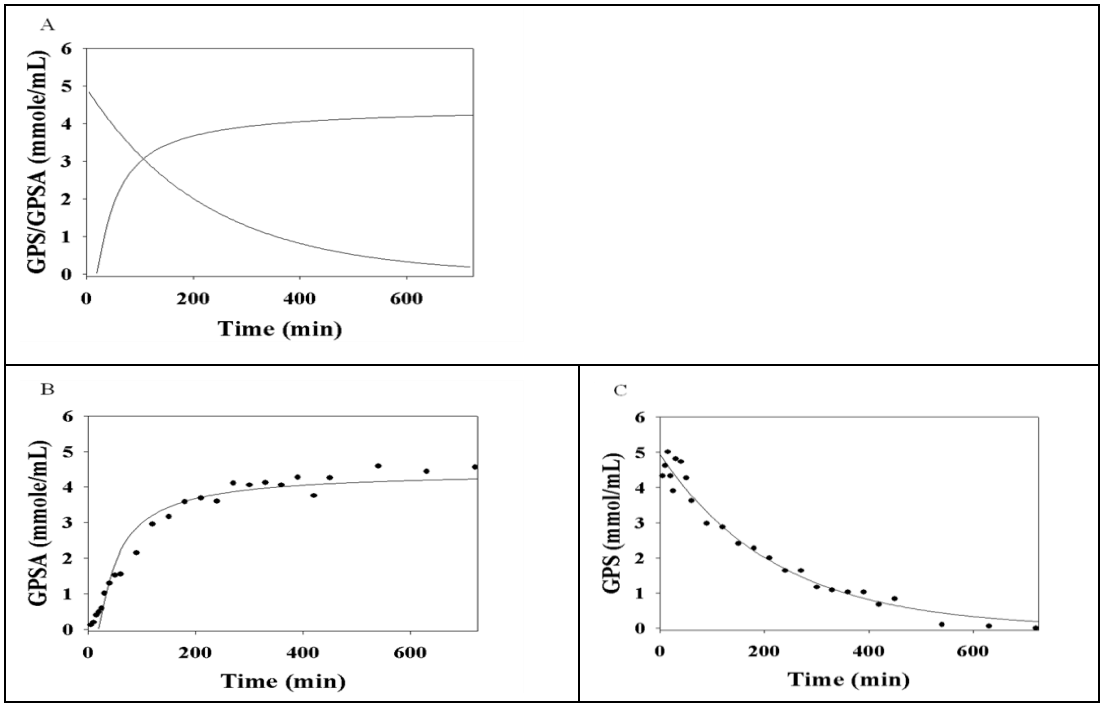


Figure 5e). Hydrolysis of GPS in 0.1M NaOH at 40°C. Reaction time: 12 h. (B) GPSA. (C) GPS.

Fig. 5. Concentration vs. reaction time profile during the transformation of geniposide into geniposidic acid. Reactions at a) 80°C. b) 70°C. c) 60°C. d) 50°C, and e) 40°C.

To accurately measure the inherent kinetics, the steady state reaction time was adopted for calculation of the kinetic parameters, i.e. 0.0-0.5 h for the reaction at 80°C, and 0.0 to1.0 h for reaction at 50 °C, and 0.0 to 3.333 h for reaction at 40 °C, etc. (Figure 5, Table 1). As seen, the hydrolytic rate (R_{hl}) of

geniposide occurred with one-to-one molar pattern regarding the formation rate (R_f) of geniposidic acid (Figure 5, Table 1), giving 8.298, 5.478, 4.320, 2.184, and 0.450 h^{-1} for R_{hl}; and 7.902, 5.478, 5.360, 2.562, and 1.452 h^{-1} for R_f, respectively, from which the pseudo-first order reaction constants (k_1') of each reaction temperature level were calculated (Equation 9, Table 1). The corresponding values of k_1' were 11.064, 8.682, 2.400, 1.021, and 0.750 h^{-1} for reaction at 80, 70, 60, 50 and 40 ^oC, respectively (Table 1).

Table 1. Reaction parameters operated at different reaction temperature[a]

Reaction temperature	80^{o}C (353 K)	70^{o}C (343 K)	60^{o}C (333 K)	50^{o}C (323 K)	40^{o}C (313 K)
Δt, h[b]	0→0.5	0→0.5	0→0.5	0→1.0	0→3.333
Variation of a, (mole L^{-1})	4.70→1.25	4.30→1.70	4.40→2.70	4.70→2.65	4.15→2.00
Variation of x, (mole L^{-1})	0→3.95	0→3.30	0→2.60	0→2.65	0→3.65
a, (mole L^{-1})	4.70	4.30	4.40	4.70	4.15
x, (mole L^{-1})	3.95	3.30	2.60	2.56	3.55
(a-x), (mole L^{-1})	0.75	1.00	1.80	2.14	0.60
Hydrolytic rate of GPS, R_{hl} (mole L^{-1} h^{-1})	8.298	5.478	4.320	2.184	0.450
Formation rate of GNPA, R_f (mole L^{-1} h^{-1})	7.902	5.478	5.760	2.562	1.452
k_1'= R_{hl}/(a-x), (h^{-1})	11.064	8.682	2.400	1.021	0.750

[a]kinetic parameters evaluated in the linear region of the reaction.

[b]Δt: reaction time interval for kinetic calculation.

3.3. Optimum cost efficient process conditions

Table 2 lists the optimum reaction time and its related percent fractional conversion that can be expected in the operation. The optimum reaction time (ORT) is defined as the maximum time point that could have steady and fast reaction kinetics, mostly showing a straight slope in the concentration–time (c-t) curve. Beyond the ORT, the reaction rate prominently slowed down. The ORT was 0.5 h at 80 ^oC and 70 ^oC. While lower reaction temperature required longer ORT like 0.833, 1.00, and 2.00 h for systems operated at 60, 50, and 40^{o}C, respectively (Table 2). Correspondingly, the expectant percent fractional conversion at each ORT was 73.4% (at 80^{o}C) and 60.5% (at 70^{o}C), respectively. While the reactions at temperature lower than 70^{o}C apparently are cost inefficient due to too long the operation time with too low percent yield (Table 2). Simultaneously,

Table 2 compares the conditions of the optimum conversion with that of 100% conversion (Table 2).

Table 2. Cost-efficient reaction time compared with the complete conversion time.

Reaction temperature T, °C	Parameters for cost-efficient conversion				
	80	70	60	50	40
T, K	353	343	333	323	313
Optimum reaction time, h (min)	0.500 (30)	0.500 (30)	0.833 (50)	1.00 (60)	2.00 (120)
Optimum percent conversion, (%)	73.4	60.5	38.6	43.6	51.8
	Time required for 100 % conversion				
100% complete conversion time, h (min)	1.50 (90)	2.333 (140)	4.017 (275)	9.00 (540)	10.00 (600)

3.4. The activation energy, E

The calculated reciprocal reaction temperatures T (K^{-1}) ($10^3\times$) were 2.83, 2.92, 3.00, 3.10, and 3.20 (K^{-1}) and the corresponding pseudo-first order kinetic constants (in $\ell nk_1'$) were 2.4073, 2.1613, 0.875, 0.0021, and -0.2877, converted to natural logarithm to obtain 1.0440, 0.9387, 0.3802, 0.0010, and-0.1250, respectively (Table 3). A plot of $\log k_1'$ vs. $1/T(K^{-1})$ yielded a straight line which showed a slope (-*E*/R) of -0.4572×10^3K (Figure 6).

Table 3. Arrhenius parameters required for calculation of activation energy

Tr, °C	80	70	60	50	40
Tr, K	353	343	333	323	313
1/T, K^{-1}	0.00283	0.00292	0.00300	0.00310	0.00320
10^3/T, (K^{-1})	2.83	2.92	3.00	3.10	3.20
k_1'= R/(a-x), (h^{-1})	11.064	8.682	2.400	1.021	0.750
$\ell nk_1'$	2.4037	2.1613	0.8755	0.0021	-0.2877
$\log k_1'$	1.0440	0.9387	0.3802	0.0010	-0.1250

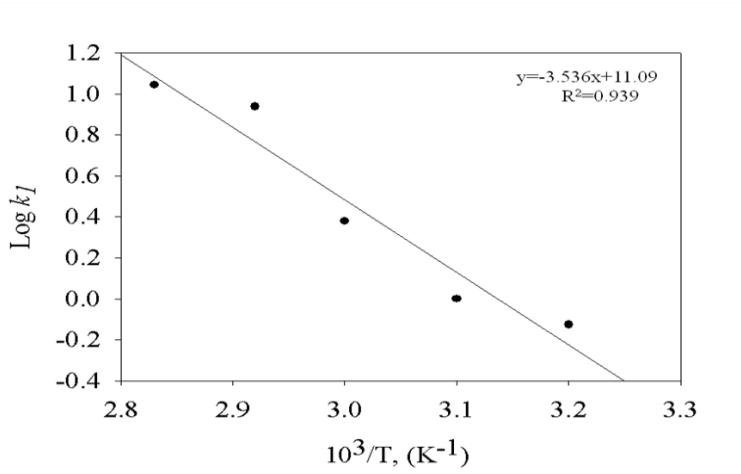


Figure 6. Plot of $\log k_1'$ vs. 1/T (K^{-1}) to evaluate the activation energy. The slope of this line is -0.4572×10^3K. The energy of activation (*E*) is the slope multiplied by -19.14 $JK^{-1}mol^{-1}$: *E* = (-19.14) × (-0.4572×10^3) = 8.751 kJ mol^{-1}.

Thus from Figure 6, the value of E could be calculated from the slope -*E*/R of the Arrhenius equation (Equation 15), i.e. by multiplication the slope with -19.14 $JK^{-1}mol^{-1}$, the energy of activation (*E*) was obtained:

E = (-19.14) × (-0.4572×10^3) = 8.751 kJ mol^{-1}

Obviously, this amount of energy of activation is rather small compared to the common chemical reactions that usually exhibit a range within 20-315 kJ mol^{-1}.

(e.g. $CO_2 + OH^- \rightarrow HCO_3$; *A* = 1e11, *Ea* = 315 kJ/mol, depicted from CATC Home Page), implicating the alkaline hydrolysis of geniposide could easily undergo to the right hand side (i.e. geniposidic acid). Conversely, the reverse reaction to the left side will be also comparably easy.

IV. CONCLUSIONS

The alkaline hydrolysis of geniposide to produce geniposidic acid obeys the pseudo-first order kinetic equation. The pseudo-first order kinetic constant (k_1') is temperature dependent, showing the values of 11.064, 8.682, 2.400, 1.021, and 0.750 h^{-1} for reactions operated at 80, 70, 60, 50 and 40 °C, respectively. The activation energy of this hydrolytic reaction is very low exhibiting only a value of 8.751 kJ mol^{-1}, implicating easy reverse reaction. The optimum reaction temperature and reaction time are 80 °C and 70 °C, and 0.50 h, and the corresponding percent fractional conversions are 73.4 and 60.5%. Based on this inherent chemical reaction parameter, the scale-up of process reactor can be easily furnished provided the parameters regarding the mass transfer and mixing are available.

V. Acknowledgement

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