Chapter III

Results and Discussions

The results of this study can be divided into 2 parts: part I and part II. Part I includes results and discussions on API-MS optimization. This part was experimented in order to get the new analytical tools to perform the aspartame degradation chemistry and aspartame stability study in part II. Part II was devoted to the aspartame stability and its degradation chemistry.

PART I: API-MS OPTIMIZATION FOR DETECTION OF ASPARTAME AND ITS DEGRADATION PRODUCTS.

In this study the API-MS technique was used to detect aspartame and its degradation products quanlitatively and quntitatively. As mentioned previously that there are two API techniques (ESI and APCI) tested in this study. The APCI was first optimized to get the optimum condition. Then optimization of ESI was done. The resulted optimum conditions of the two techniques were then used to detect aspartame and its degradation products. The results of the two techniques were compared and the better technique was used for the rest of the study (Part II).

1. **Optimization of APCI parameters**

1.1 **Cone Voltage** In this study, APCI gave the M+1 peak of protonated aspartame at M/Z = 295 for all the cone voltage values tested (Figure 3.1).

As shown in Figure 3.1, it is obvious that the cone voltage affects fragmentation pattern of aspartame. The higher cone voltage induced more fragmentation of the protonated aspartame. This can be explained by the theory of collision induced fragmentation which states that the higher cone voltage causes higher velocity of charged particles. The higher velocity results in more powerful collision among particles within the ionization chamber yielding more bond dissociation. One normally expects that maximum (M+1) peak intensity would be at the minimal fragmentation. From Figure 3.2, it is clear that maximum molecular peak intensity was at the cone voltage of 60 V. This voltage probably was the balance between minimal fragmentation and effective ionization (collision between sample molecules and solvent ions).

1.2 **APCI Discharge Needle Voltage** Figure 3.3 shows chromatograms of aspartame obtained at various discharge needle voltages (SIR at M/Z = 295). The results indicate that the discharge needle voltage of 3.50 kV provided the best detection of aspartame molecular ions.

This voltage was applied to corona discharge needle to ionize the solvent molecules in order to generate reagent ions those when collide with sample molecules give the sample ions through charge exchange reactions.

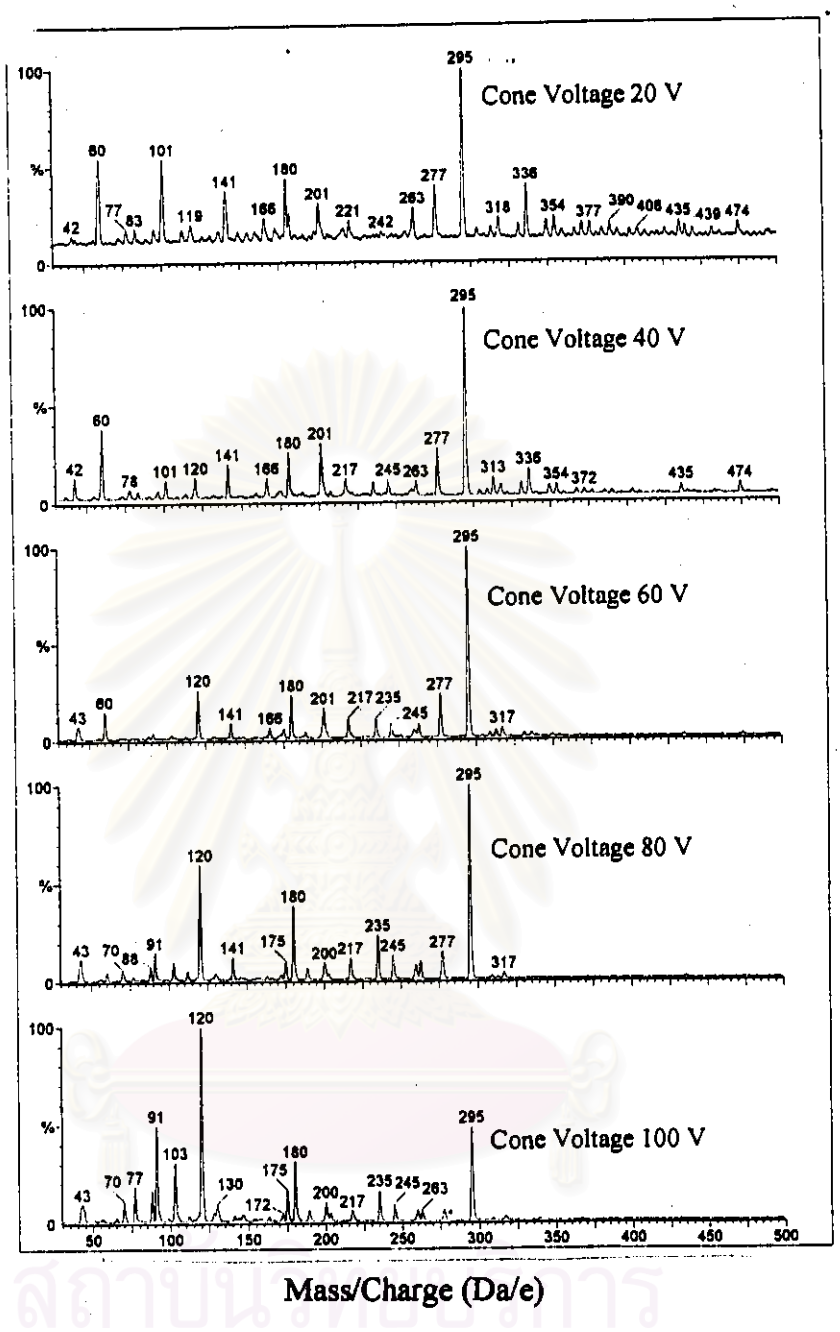


Figure 3.1 Fragmentation Pattern of aspartame at different cone voltage by APCI.

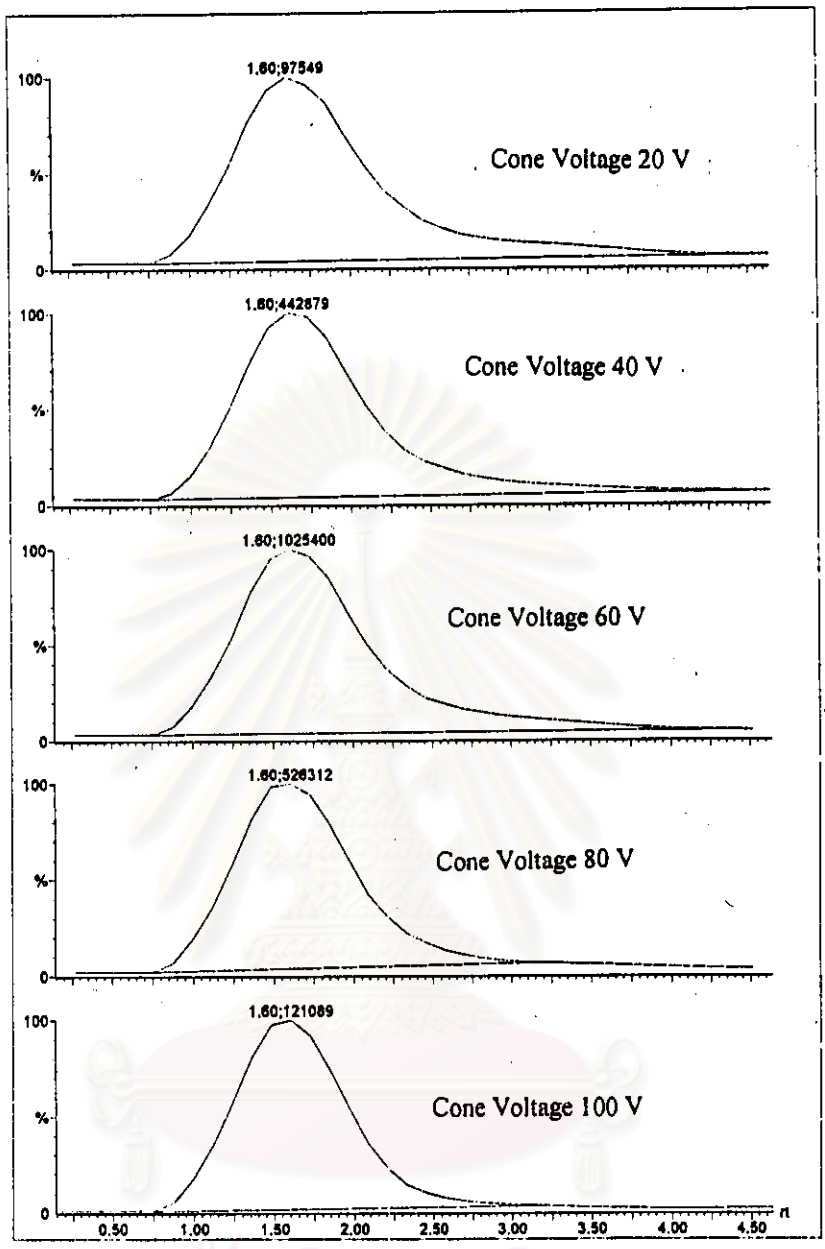


Figure 3.2 SIR signal (at M/Z = 295) of 100 ppm aspartame at various cone voltages under APCI. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 250 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.20 kV	Source temperature : 120 ^{0}C

$$S \xrightarrow{\text{High Voltage}} S^+$$

$$S^+ + M \xrightarrow{\text{Charge Exchange}} S + M^+ \quad ; S = \text{Solvent molecule}, \; M = \text{Sample molecule}$$

In this, the optimal needle voltage of 3.50 kV is probably the best voltage that gives enough solvent ions to collide with aspartame molecule. Too high of this voltage could cause some fragmentation of the interested ions and therefore, decreases the sensitivity of the detection.

1.3 **Counter Electrode Voltage** In this case, the optimal voltage is the voltage that gives maximum molecular ions of aspartame. Figure 3.4 shows chromatograms of aspartame at various counter electrode voltage (SIR at M/Z = 295). The results indicate the optimal voltage of 0.40 kV.

This voltage spans the area from the tip of sample probe to the counter electrode and, therefore, corresponds to the speed of ions in this area (see Figure 1.8). Higher voltage gradient will increase speed of ions and causes more collision.

1.4 **Lens2 Voltage** Optimization to get the maximum of aspartame molecular ion was done (Figure 3.5). Results show that lens 2 voltage of 240 V was the optimal value. This voltage is the voltage that spans the area between skimmer and differential aperture. It affects directly to the speed of ions passing through that region and, therefore, affects the collision among particles. The optimal value of 240 V was, therefore, the balance between maximum ability to direct the ions into the mass analyzer and the minimum fragmentation of the interested ions.

1.5 **Lens3 Voltage** Figure 3.6 shows chromatograms of aspartame (SIR at M/Z = 295) at various lens 3 voltages. Results show that lens 3 voltage of 10 V was

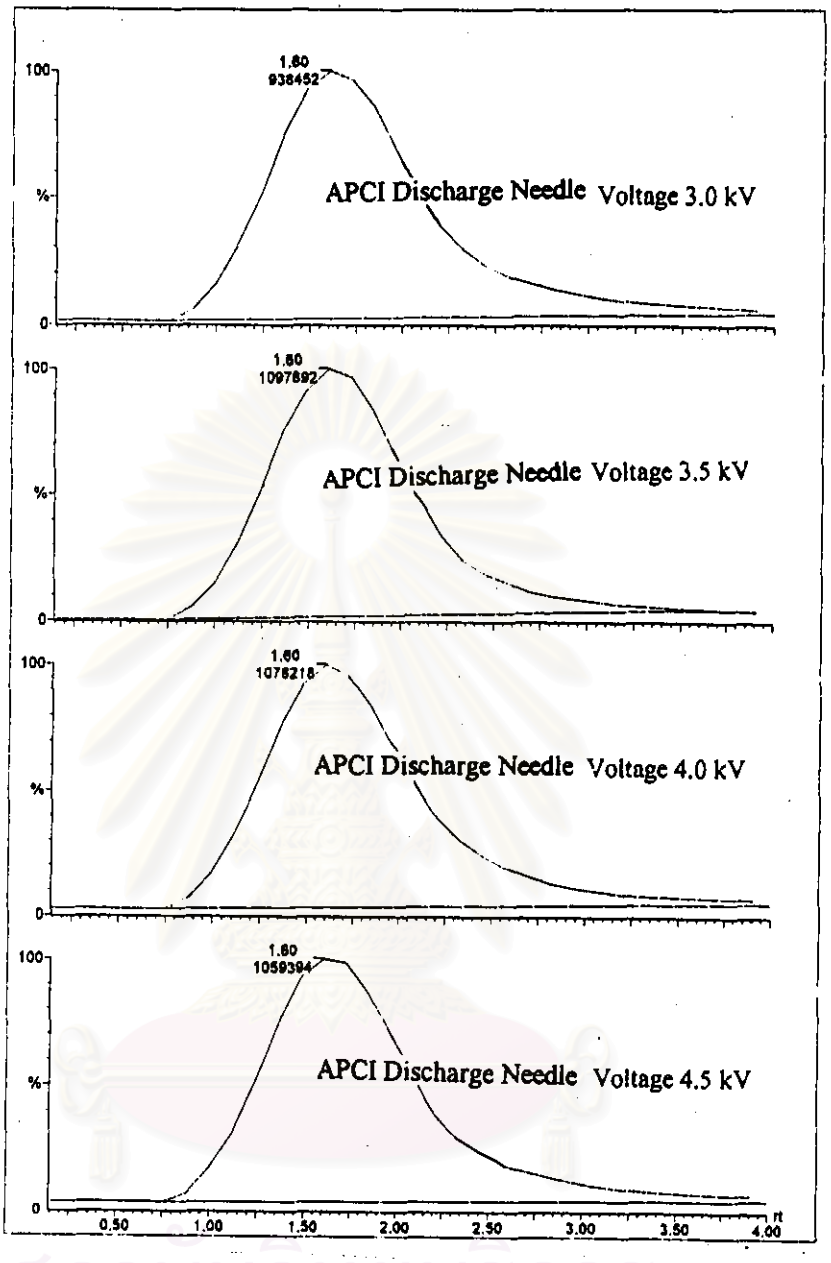


Figure 3.3 SIR signal (at M/Z = 295) of 100 ppm aspartame at various APCI discharge needle voltages obtained under APCI. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 250 V
Counter electrode voltage : 0.20 kV	Lens 3 voltage : 10 V
Cone voltage : 60 V	Source temperature : 120 ^{o}C

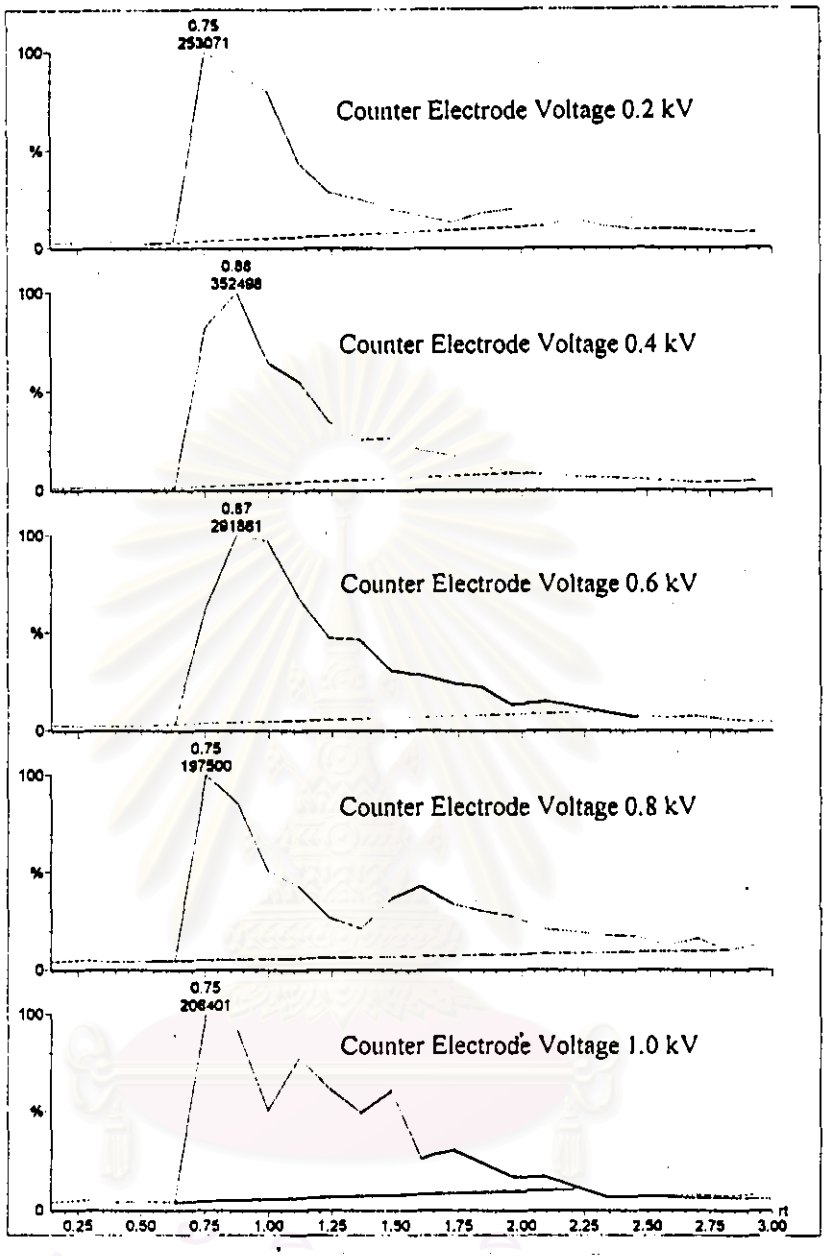


Figure 3.4 SIR signal (at M/Z = 295) of 100 ppm aspartame at various counter electrode voltages obtained under APCI. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 250 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Cone voltage : 60 V	Source temperature : 120 ^{0}C

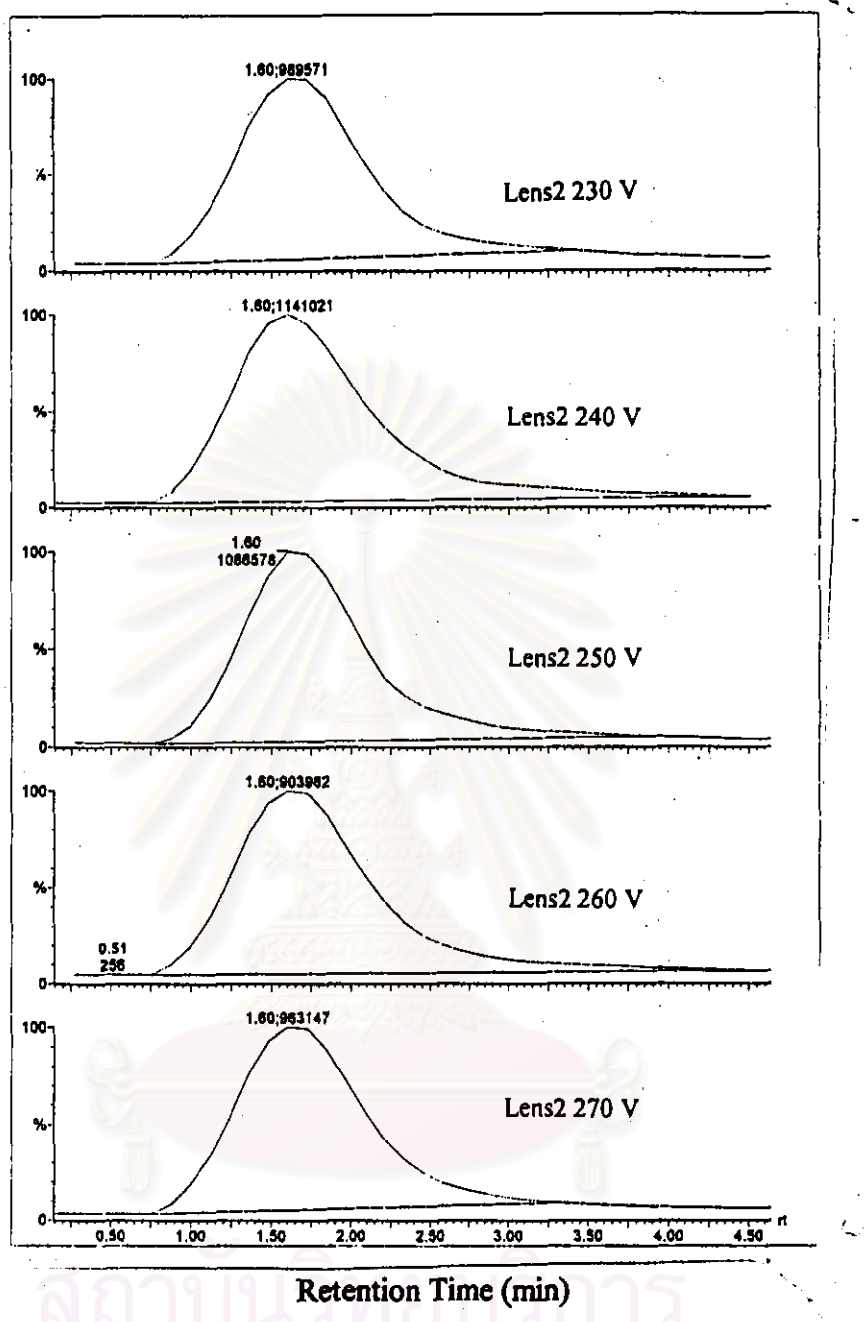


Figure 3.5 SIR signal (at M/Z = 295) of 100 ppm aspartame at various lens 2 voltages obtained under APCI. All other parameters were as follows:

Flow rate : 0.5 mL/min	Cone voltage : 60 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C

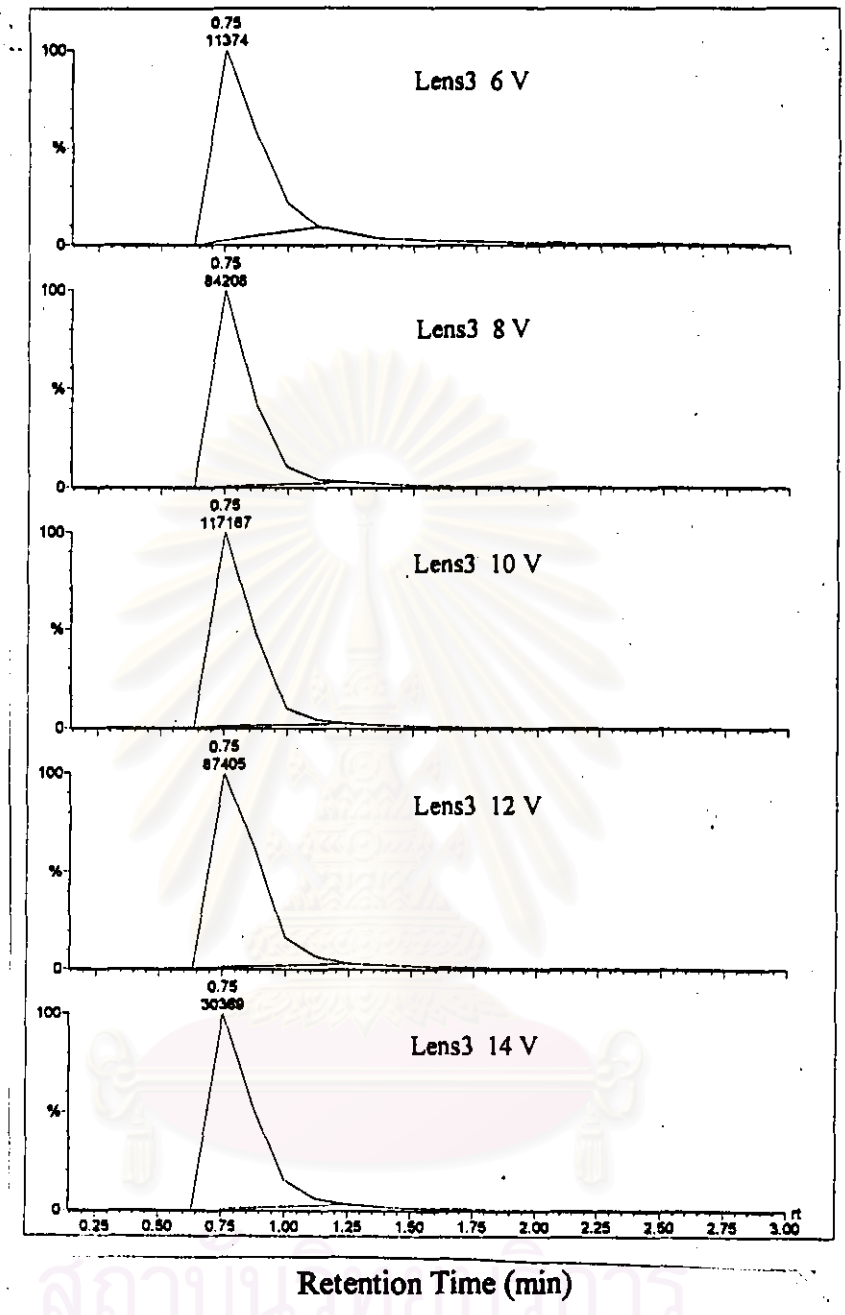


Figure 3.6 SIR signal (at M/Z = 295) of 100 ppm aspartame at various lens 3 voltages obtained under APCI. All other parameters were as follows:

Flow rate : 0.5 mL/min	Cone voltage : 60 V
Corona discharge pin voltage : 3.50 kV	Lens 2 voltage : 240 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C

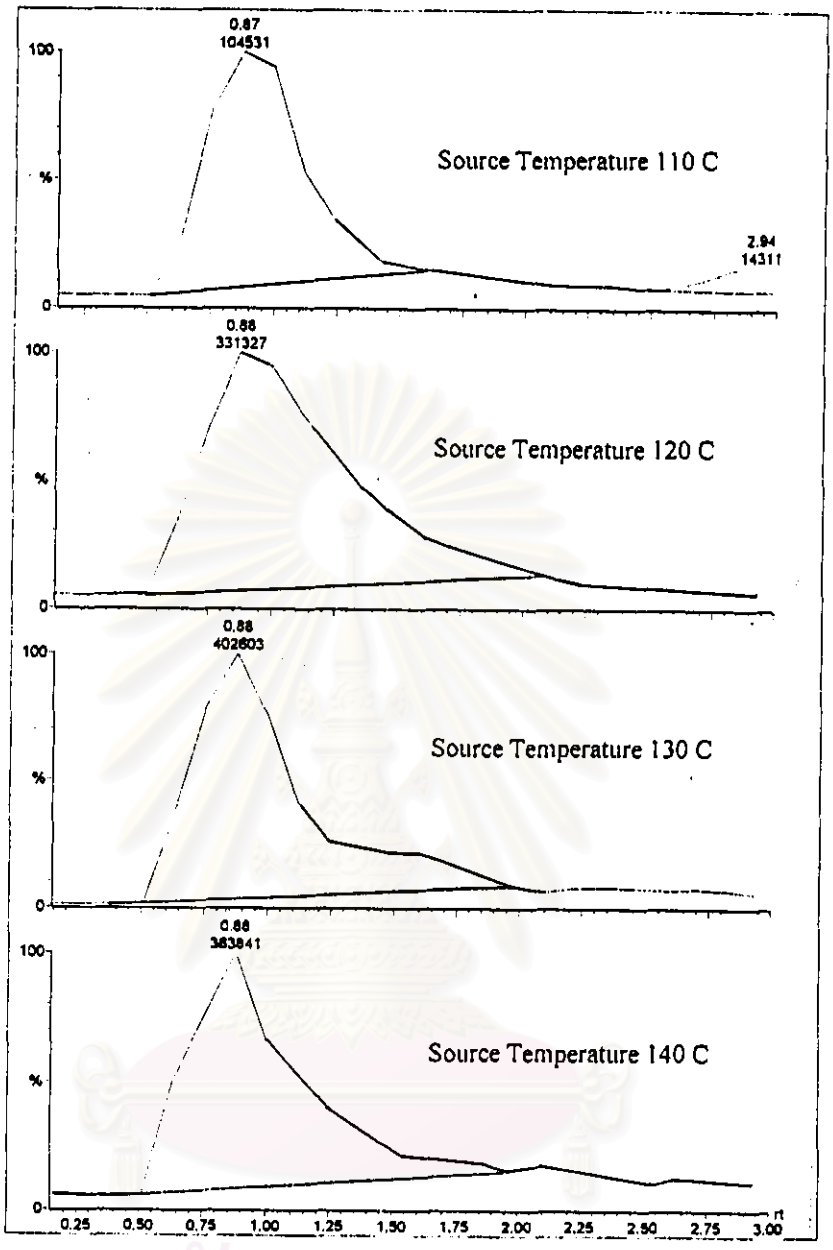


Figure 3.7 SIR signal (at M/Z = 295) of 100 ppm aspartame at various source temperatures obtained under APCI. All other parameters were as follows:

Flow rate : 0.5 mL/min	Cone voltage : 60 V
Corona discharge pin voltage : 3.50 kV	Lens 2 voltage : 240 V
Counter electrode voltage : 0.40 kV	Lens 3 voltage : 10 V

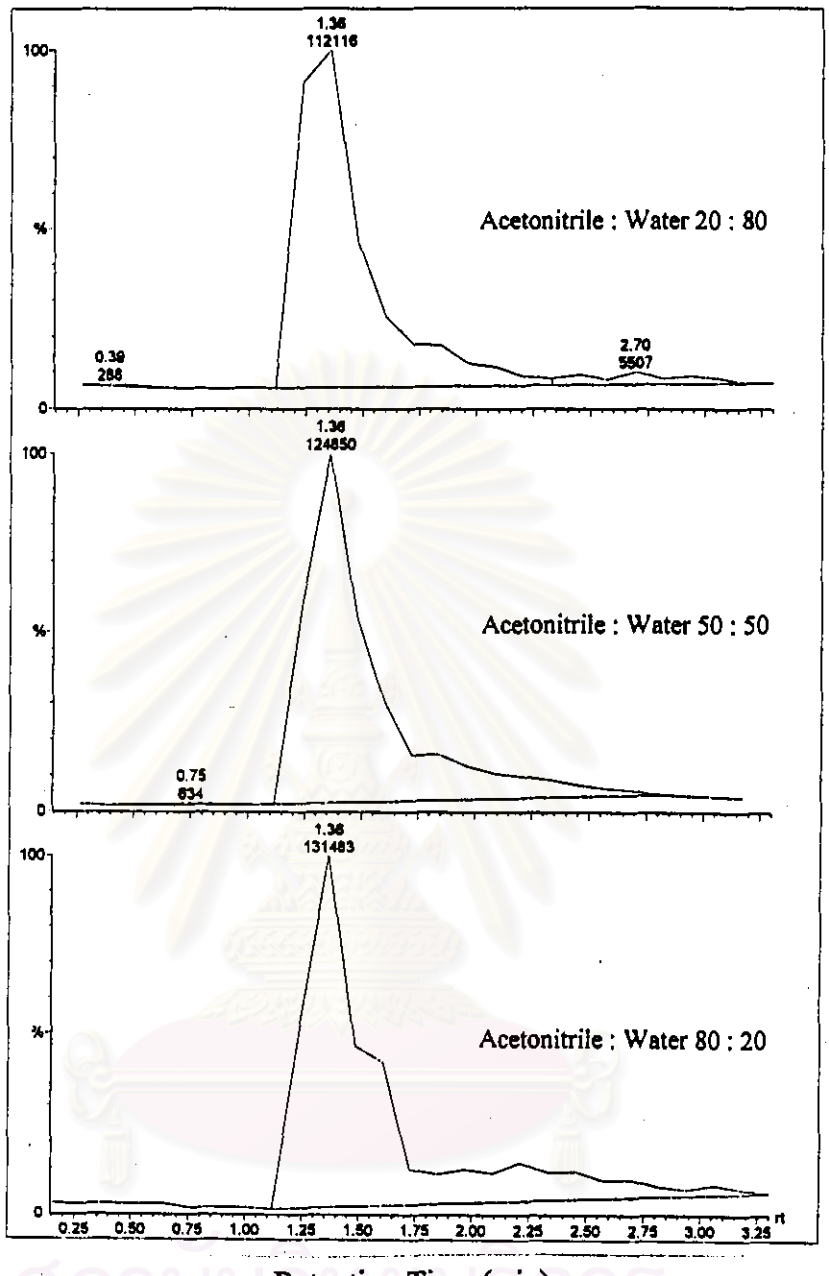


Figure 3.8 SIR signal (at M/Z = 295) of 100 ppm aspartame obtained under various mobile phase components. All other parameters were as follows:

Corona discharge pin voltage : 3.50 kV	Lens 2 voltage : 240 V
Counter electrode voltage : 0.40 kV	Lens 3 voltage : 10 V
Cone voltage : 60 V	Source temperature : 120 ^{0}C

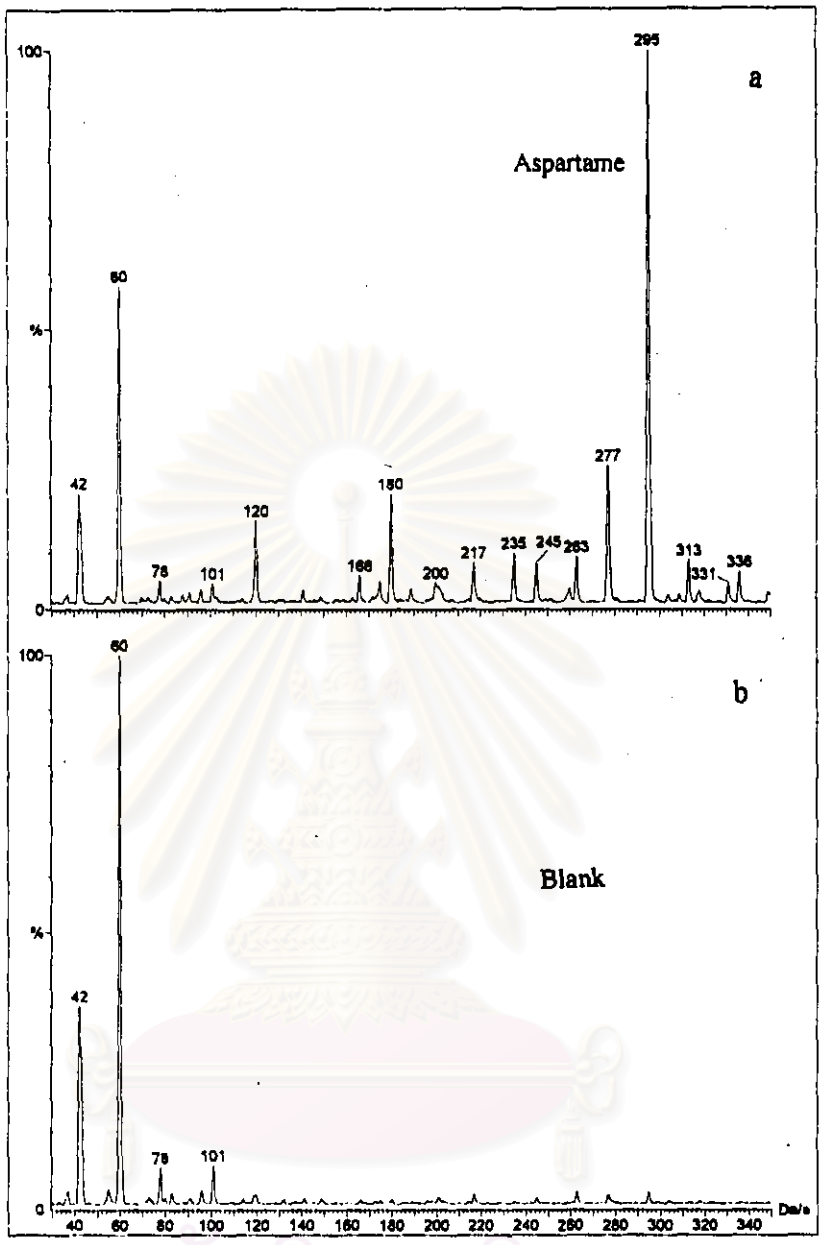


Figure 3.9 APCI mass spectrum of a) 1000 ppm aspartame and b) mobile phase under optimal condition.

Flow rate : 0.5 mL/min

Corona discharge pin voltage : 3.50 kV

Counter electrode voltage : 0.40 kV

Cone voltage : 60 V

Lens 2 voltage : 240 V

Lens 3 voltage : 10 V

Source temperature : 120 ^{0}C

the optimal value. This voltage is the voltage that spans the area between differential aperture and the begining of mass analyzer. The explanation for the optimal value of 10 V is in the same analogy to that of lens 2.

1.6 **Source Temperature** For APCI, the optimal source temperature was 120 ^{0}C (Figure 3.7). Although source temperature of 130 ^{0}C gave higher peak area for aspartame molecular ion, the signal was much more fluctuated. At 120 ^{0}C, the signal was quite stable, therefore, it was used for the rest of experiments.

1.7 **Mobile Phase** In this study, a mixture of acetonitrile and water was used as a mobile phase. Since ratio of acetonitrile to water did not affect the peak area of aspartame molecular ion (Figure 3.8). The 20:80 (V/V) acetonitrile:water was used as the mobile phase in order to minimize the use of organic solvent.

1.8 **Flow Rate** The optimal flow rate for APCI was 0.5 mL/min. This rate gave a stable and prominent signal together with the acceptable time required for each injection.

Figure 3.9a shows APCI mass spectrum of 50 μL of 1000 ppm aspartame obtained under optimal condition, i.e., cone voltage of 60 V, corona discharge needle voltage of 3.5 kV, counter electrode voltage of 0.2 kV, lens 2 voltage of 240 V, lens 3 voltage of 10 V, source temperature of 120 ^{0}C and the flow rate of 0.5 mL/min. Figure 3.9b shows spectrum obtained under the same condition as performed in Figure 3.9a except that no aspartame solution was injected, only mobile phase was passed into the ionization source. By comparing Figure 3.9a and 3.9b, it is obvious that peaks at M/Z of 42,60,78 and 101 represent cluster ions of $(CH_3CN)H^+$, $(CH_3CN\bullet H_2O)H^+$, $(CH_3CN\bullet 2H_2O)H^+$ and $(2CH_3CN\bullet H_2O)H^+$, respectively. Peaks at M/Z of

120, 166, 180, 217, 235, 245, 263 and 277 represent fragmented ions occurred during ionization process. Structures of these fragmented ions are shown in Table 3.1.

Table 3.1 Some fragmented ions present in APCI mass spectrum of aspartame obtained under optimal condition.

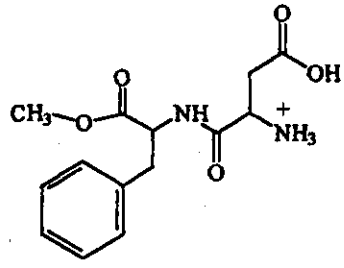
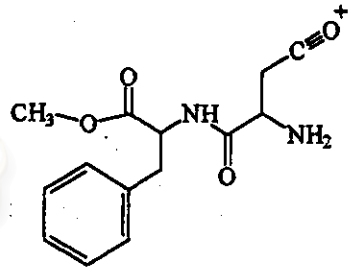
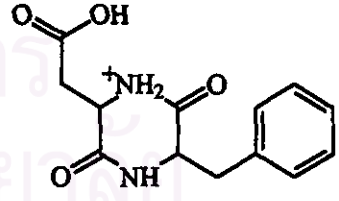
M/Z	Ions	Structures
295	M+1	 Protonated Aspartame
277	(M+1)-18	
263	(M+1)-32	 Protonated Diketopiperazine

Table 3.1 (Continue)

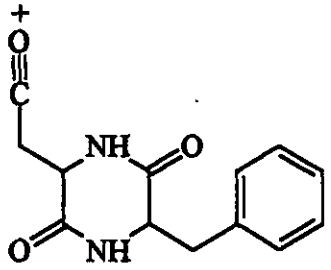
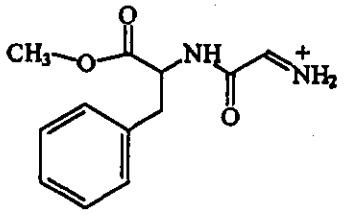
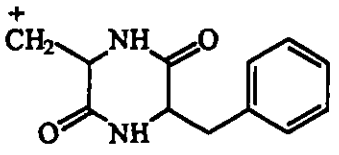
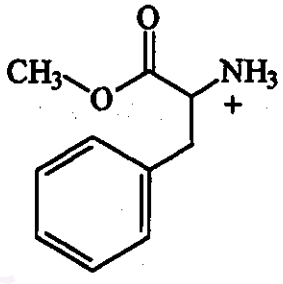
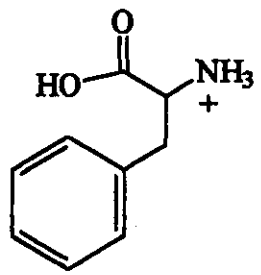
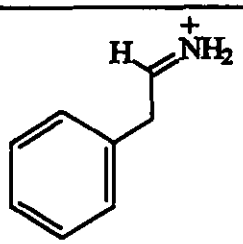
M/Z	Ions	Structures
245	263-18	
235	(M+1)-60	
217	245-28	
180	(M+1)-115	 Protonated Phenylalanine methyl ester

Table 3.1 (Continue)

M/Z	Ions	Structures
166	(M+1)-129	 Protonated Phenylalanine
120	180-60	

Mass spectrum of all six aspartame degradation products were obtained under the optimal condition. Figure 3.10 - 3.15 show mass spectrum of those compounds, i.e., aspartic acid, aspartyl phenylalanine, diketopiperazine, methanol, phenylalanine and phenylalanine methyl ester, respectively. Elucidation of peaks present in each spectrum reveals structures of various fragmented ions as shown in Table 3.2 for aspartic acid spectrum, Table 3.3 for aspartyl phenylalanine spectrum, Table 3.4 for diketopiperazine spectrum, Table 3.5 for methanol spectrum, Table 3.6 for phenylalanine spectrum and Table 3.7 for phenylalanine methyl ester spectrum.

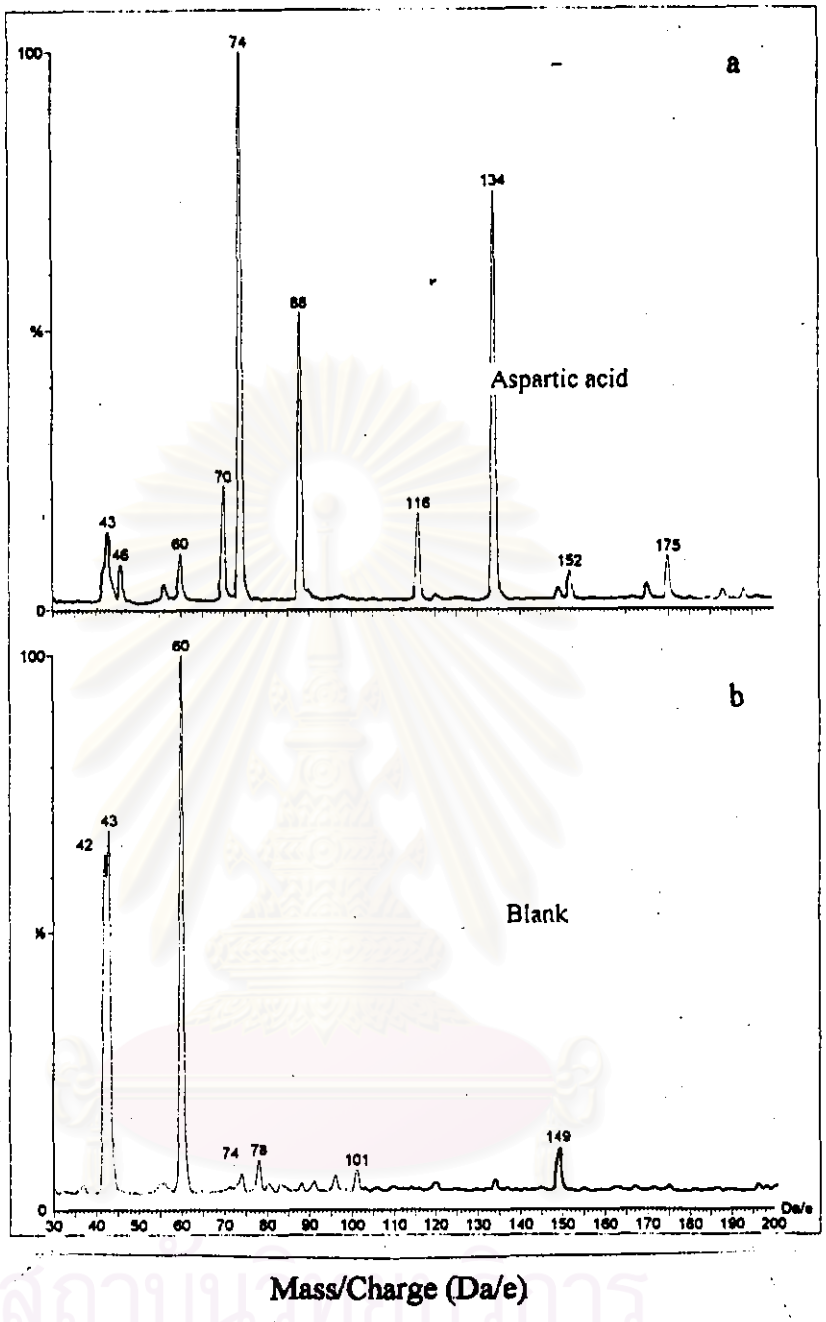


Figure 3.10 APCI mass spectrum of a) 850 ppm aspartic acid and b) mobile phase under optimal condition.

Flow rate : 0.5 mL/min

Corona discharge pin voltage : 3.50 kV

Counter electrode voltage : 0.40 kV

Cone voltage : 60 V

Lens 2 voltage : 240 V

Lens 3 voltage : 10 V

Source temperature : 120 ^{0}C

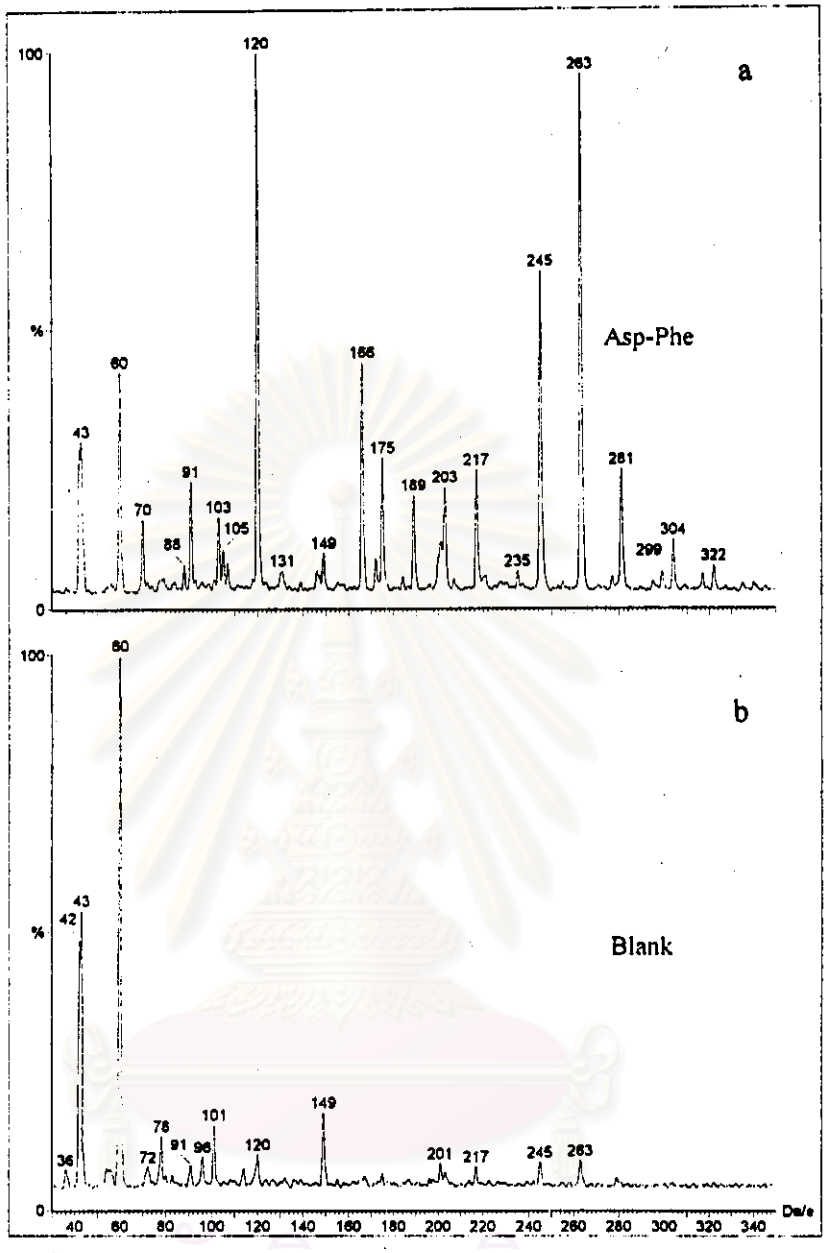


Figure 3.11 APCI mass spectrum of a) 290 ppm aspartyl phenylalanine and b) mobile phase under optimal condition.

Flow rate : 0.5 mL/min

Corona discharge pin voltage : 3.50 kV

Counter electrode voltage : 0.40 kV

Cone voltage : 60 V

Lens 2 voltage : 240 V

Lens 3 voltage : 10 V

Source temperature : 120 ^{0}C

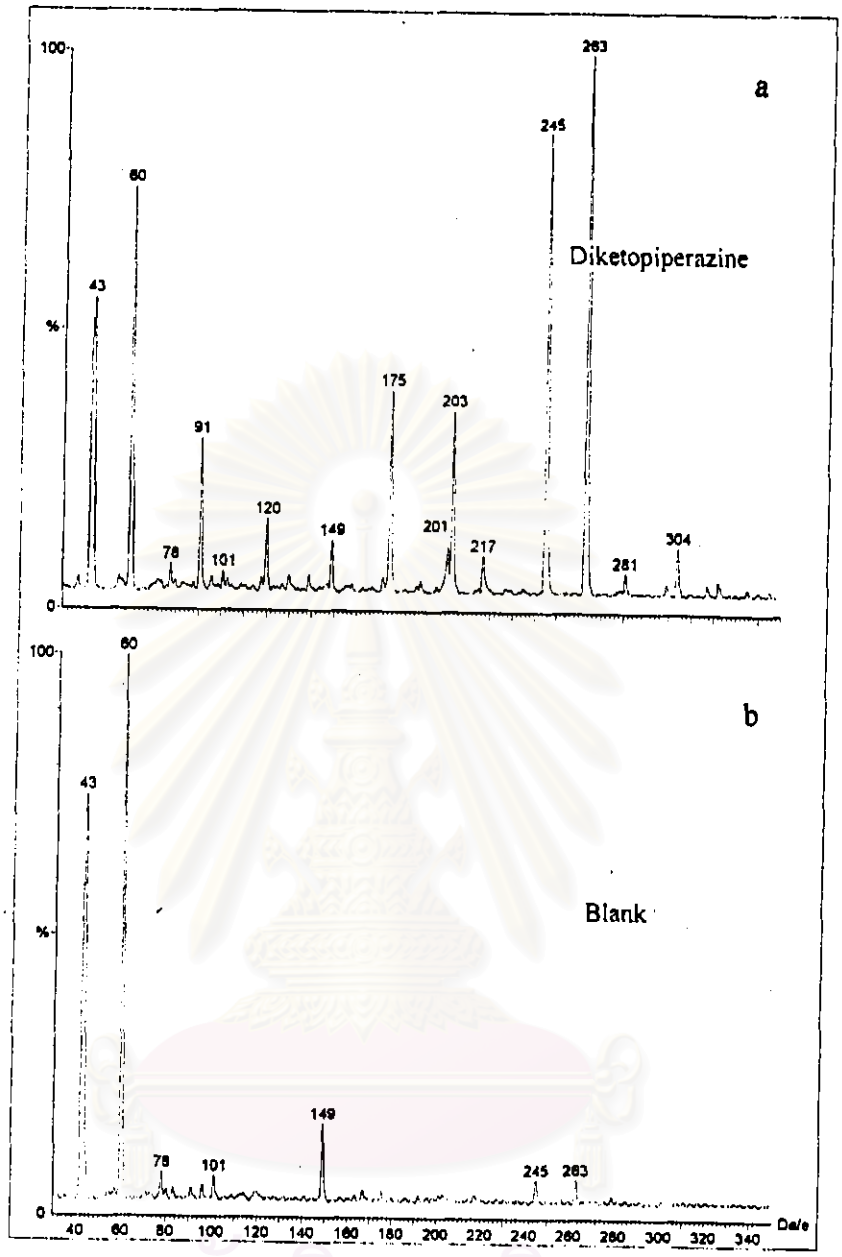


Figure 3.12 APCI mass spectrum of a) 320 ppm diketopiperazine and b) mobile phase under optimal condition.

Flow rate : 0.5 mL/min

Corona discharge pin voltage : 3.50 kV

Counter electrode voltage : 0.40 kV

Cone voltage : 60 V

Lens 2 voltage : 240 V

Lens 3 voltage : 10 V

Source temperature : 120 ^{0}C

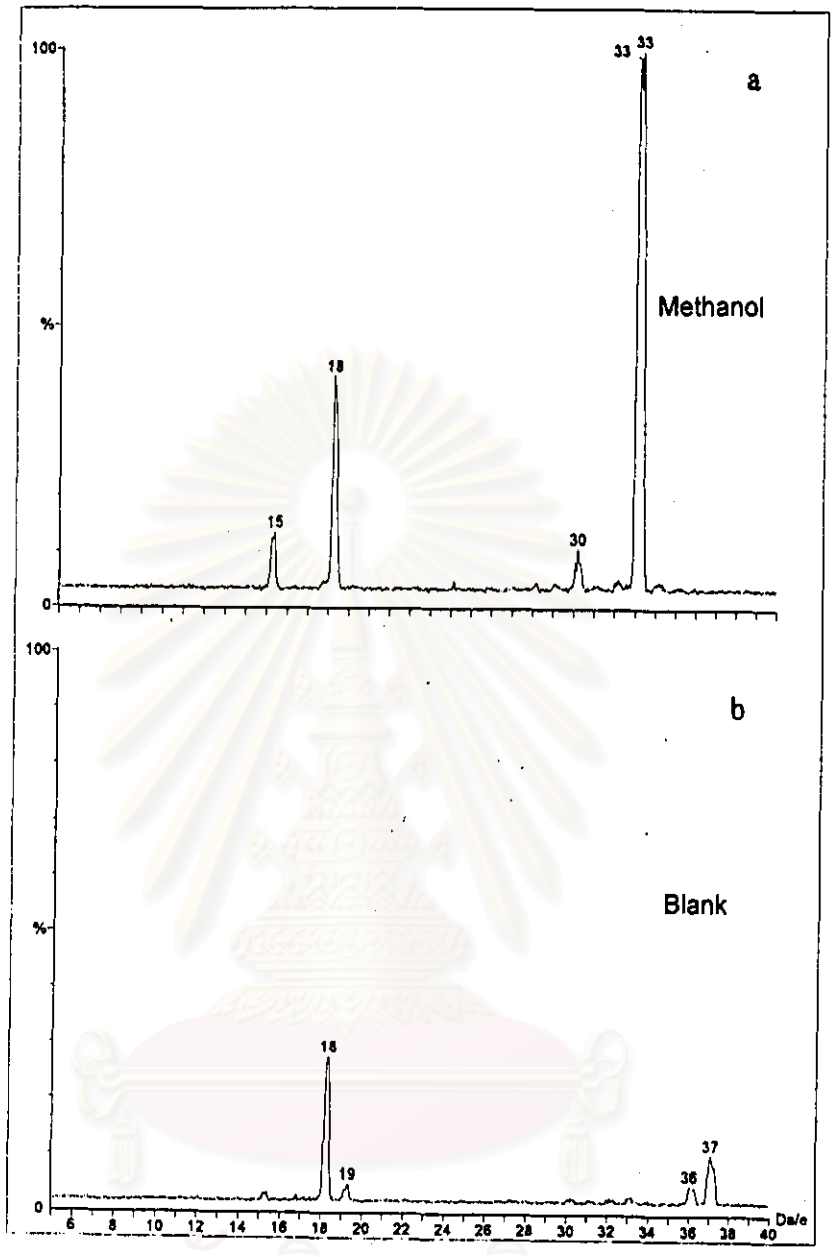


Figure 3.13 APCI mass spectrum of a) absolute methanol and b) mobile phase under optimal condition.

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

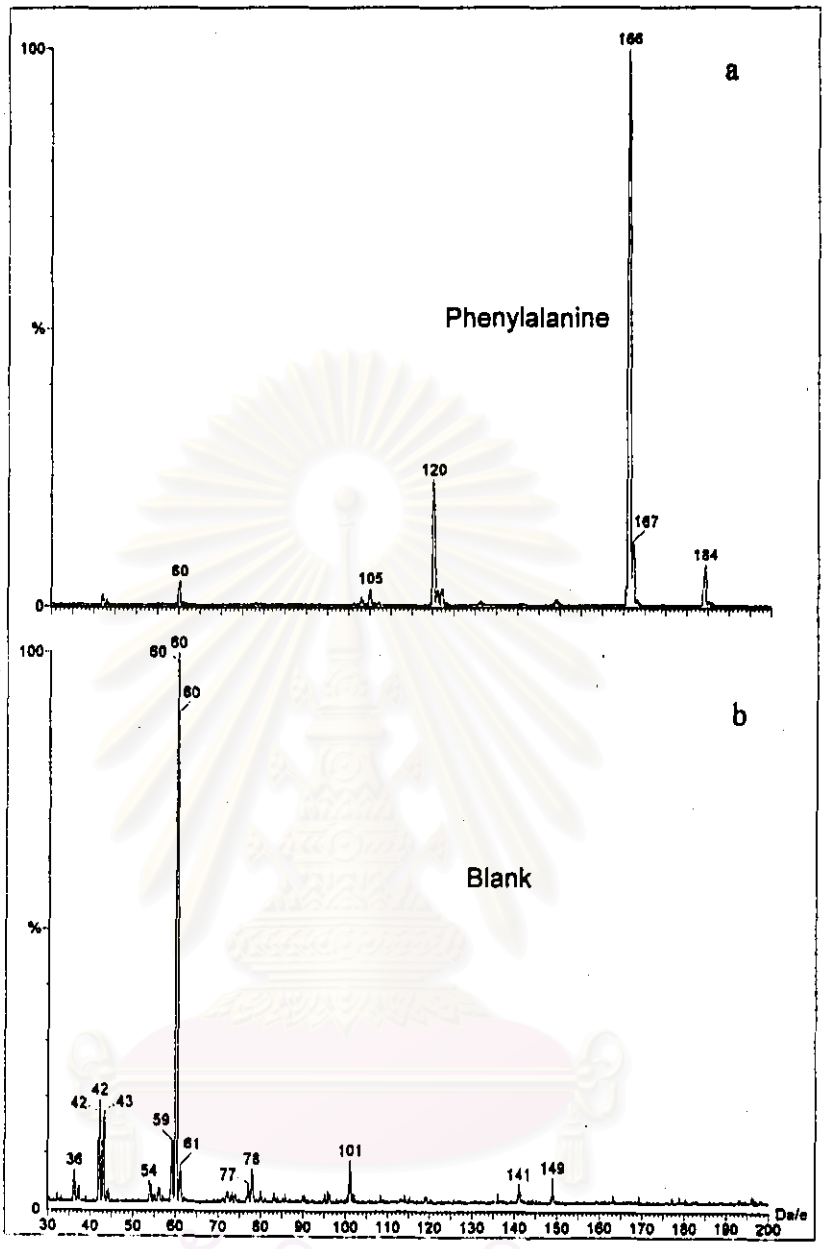


Figure 3.14 APCI mass spectrum of a) 1000 ppm phenylalanine and b) mobile phase under optimal condition.

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

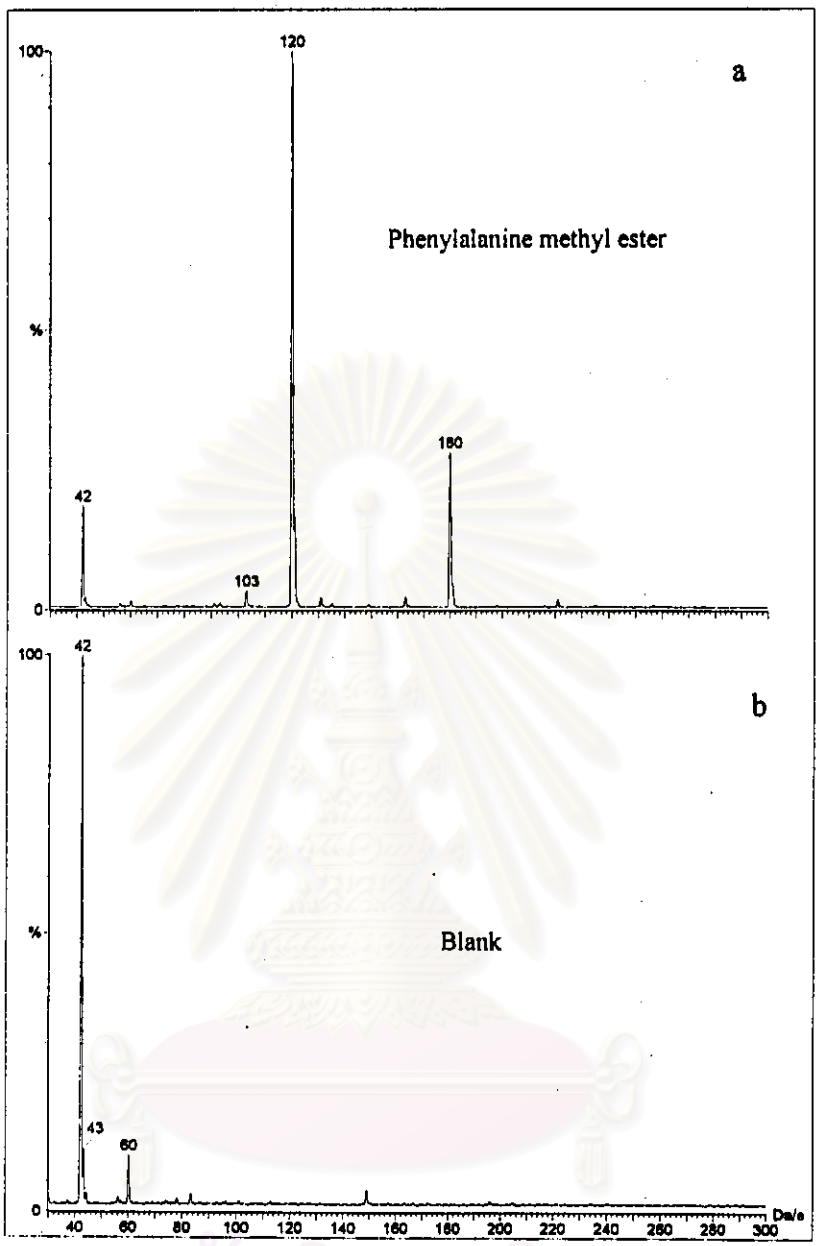


Figure 3.15 APCI mass spectrum of a) 940 ppm phenylalanine methyl ester and b) mobile phase under optimal condition.

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

Table 3.2 Some fragmented ions present in APCI mass spectrum of aspartic acid obtained under optimal condition.

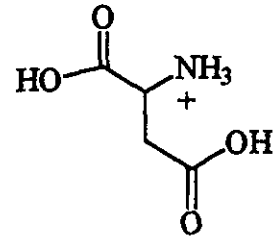
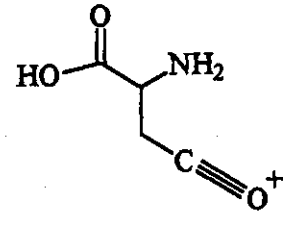
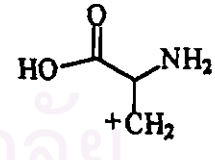
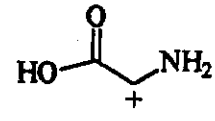
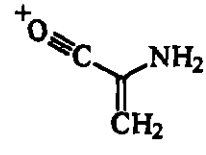
M/Z	Ions	Structures
175	(M+1)+41	Protonated Aspartic acid $+CH_3CN$
152	(M+1)+18	Protonated Aspartic acid $+H_2O$
134	M+1	 Protonated Aspartic acid
116	(M+1)-18	
88	116-28	
74	88-14	
70	88-18	

Table 3.3 Some fragmented ions present in APCI mass spectrum of aspartyl phenylalanine obtained under optimal condition.

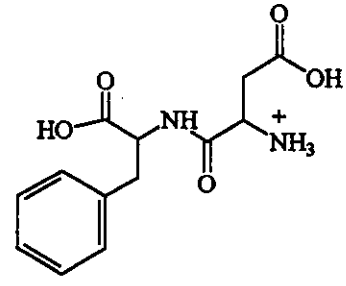
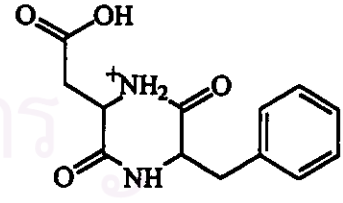
M/Z	Ions	Structures
322	(M+1)+41	Protonated Aspartyl phenylalanine $+CH_3CN$
304	(M+1)-18+41	Protonated Diketopiperazine$+CH_3CN$
281	M+1	 Protonated Aspartyl phenylalanine
263	(M+1)-18	 Protonated Diketopiperazine

Table 3.3 (Continue)

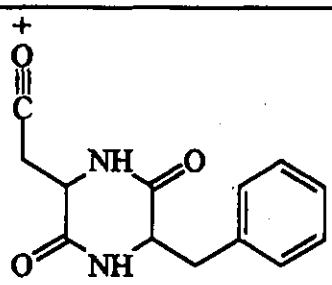
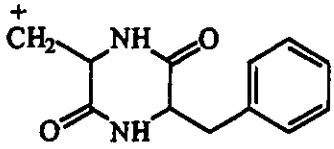
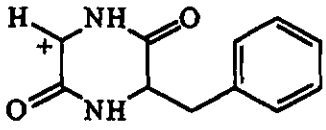
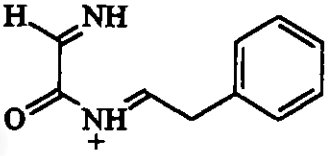
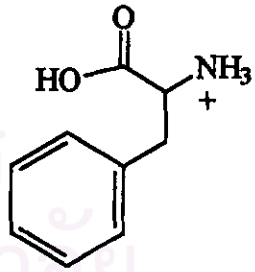
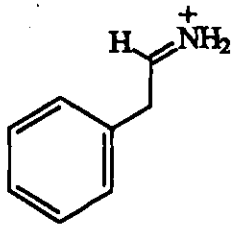
M/Z	Ions	Structures
245	263-18	
217	245-28	
203	217-14	
175	203-28	
166	(M+1)-115	 Protonated Phenylalanine
120	180-46	

Table 3.3 (Continue)

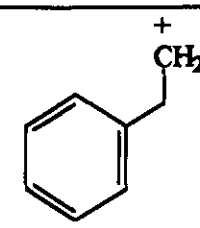
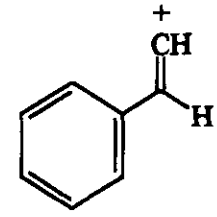
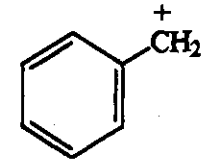
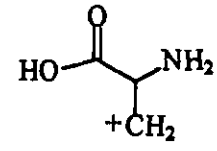
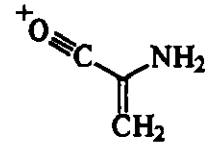
M/Z	Ions	Structures
105	(M+1)-176	
103	120-17	
91	105-14	
88	116-28	
70	88-18	

Table 3.4 Some fragmented ions present in APCI mass spectrum of diketopiperazine obtained under optimal condition.

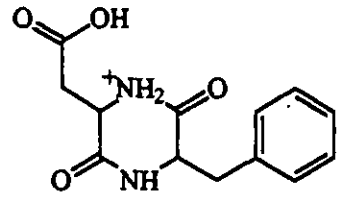
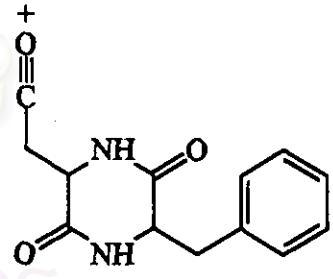
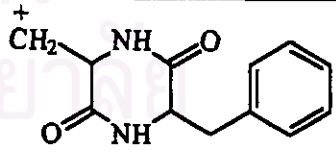
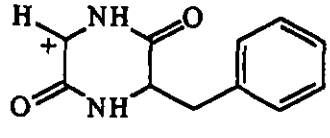
M/Z	Ions	Structures
304	(M+1)+41	Protonated Diketopiperazine+CH_3CN
281	(M+1)+18	Protonated Diketopiperazine+H_2O
263	M+1	 Protonated Diketopiperazine
245	(M+1)-18	
217	245-28	
203	217-14	

Table 3.4 (Continue)

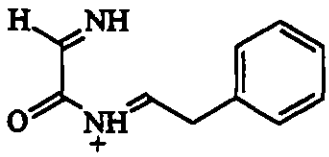
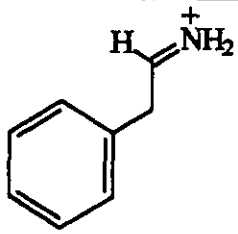
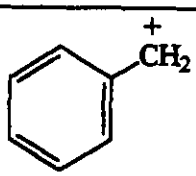
M/Z	Ions	Structures
175	203-28	
120	(M+1)-143	
91	(M+1)-172	

Table 3.5 Some fragmented ions present in APCI mass spectrum of methanol obtained under optimal condition.

M/Z	Ions	Structures
33	M+1	$CH_3OH_2^+$ Protonated Methanol
30	(M+1)-3	$HC \equiv O^+$
15	(M+1)-18	CH_3^+

Table 3.6 Some fragmented ions present in APCI mass spectrum of phenylalanine obtained under optimal condition.

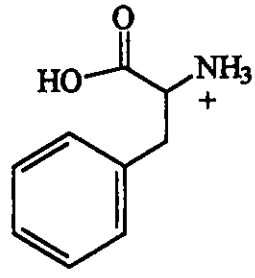
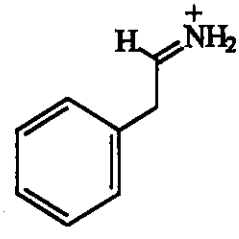
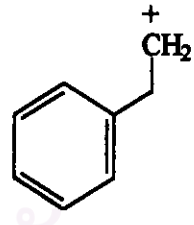
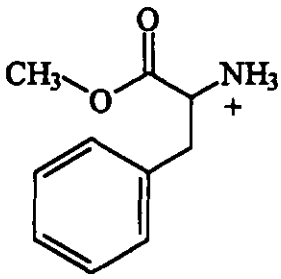
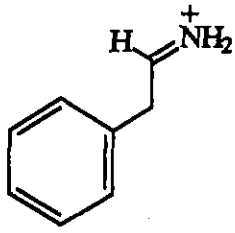
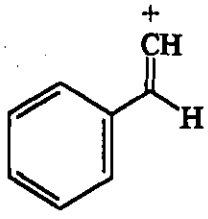
M/Z	Ions	Structures
184	(M+1)+18	Protonated Phenylalanine + H_2O
166	M+1	 Protonated Phenylalanine
120	(M+1)-46	
105	(M+1)-61	

Table 3.7 Some fragmented ions present in APCI mass spectrum of phenylalanine methyl ester obtained under optimal condition.

M/Z	Ions	Structures
180	M+1	 Protonated Phenylalanine methyl ester
120	(M+1)-60	
103	120-17	

2. **Optimization of ESI Parameters**

2.1 **Cone Voltage** ESI gave the M+1 peak of aspartame at M/Z = 295 (Figure 3.16) similar to that of APCI. From Figure 3.17 it was obvious that the cone voltage of 30 V gave the maximum peak area of protonated aspartame. Therefore, this cone voltage was the optimized voltage.

2.2 **ESI Capillary Tip Voltage** From Figure 3.18 it can be concluded that the optimal probe voltage for the analysis of aspartame by ESI was 3.5 kV.

This voltage is applied to the tip of electrospray sample probe. This voltage helps atomized sample solution into tiny droplets. The size of the droplet usually affects ionization efficiency (see details of ionization mechanism on page 12).

2.3 **Counter Electrode Voltage** Like APCI, this voltage spans the area from the probe tip to the counter electrode. Figure 3.19 shows chromatograms of aspartame (SIR at M/Z = 295) obtained under various counter electrode voltages. The results indicate the optimal voltage of 0.3 kV.

2.4 **Lens 2 Voltage** Figure 3.20 shows chromatograms of aspartame (SIR at M/Z = 295) obtained under various lens 2 voltages. It can be concluded that optimal voltage was 210 V. The role of lens 2 voltage in ESI is similar to that explained earlier in APCI (page 29).

2.5 **Lens 3 Voltage** Figure 3.21 shows chromatograms of aspartame at various lens 3 voltages. The results indicate the optimal voltage of 10 V. The role of lens 3 voltage in ESI is similar to that explained earlier in APCI (page 29).

2.6 **Source Temperature** As shown in Figure 3.22, the peak area of aspartame was increased along with the increase in source temperature. The source

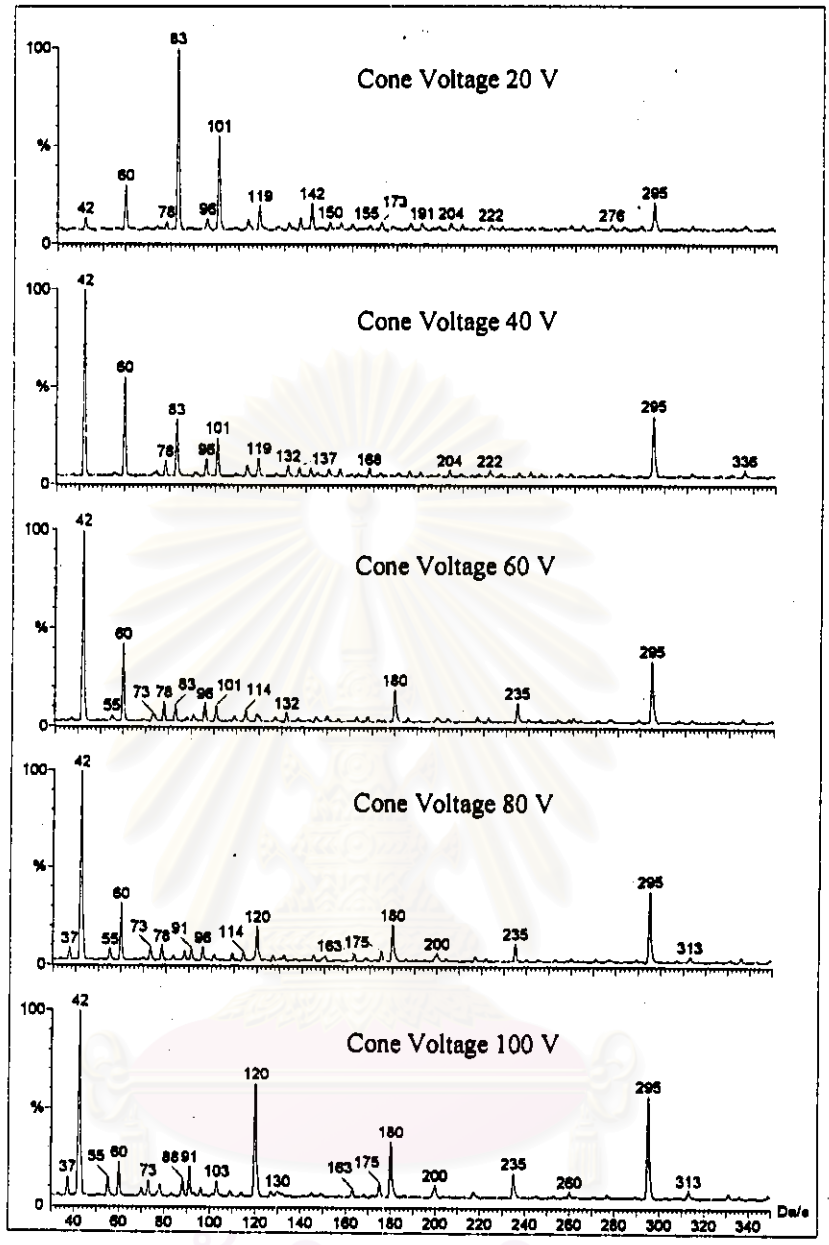


Figure 3.16 Fragmentation Pattern of aspartame at different cone voltage by ESI.

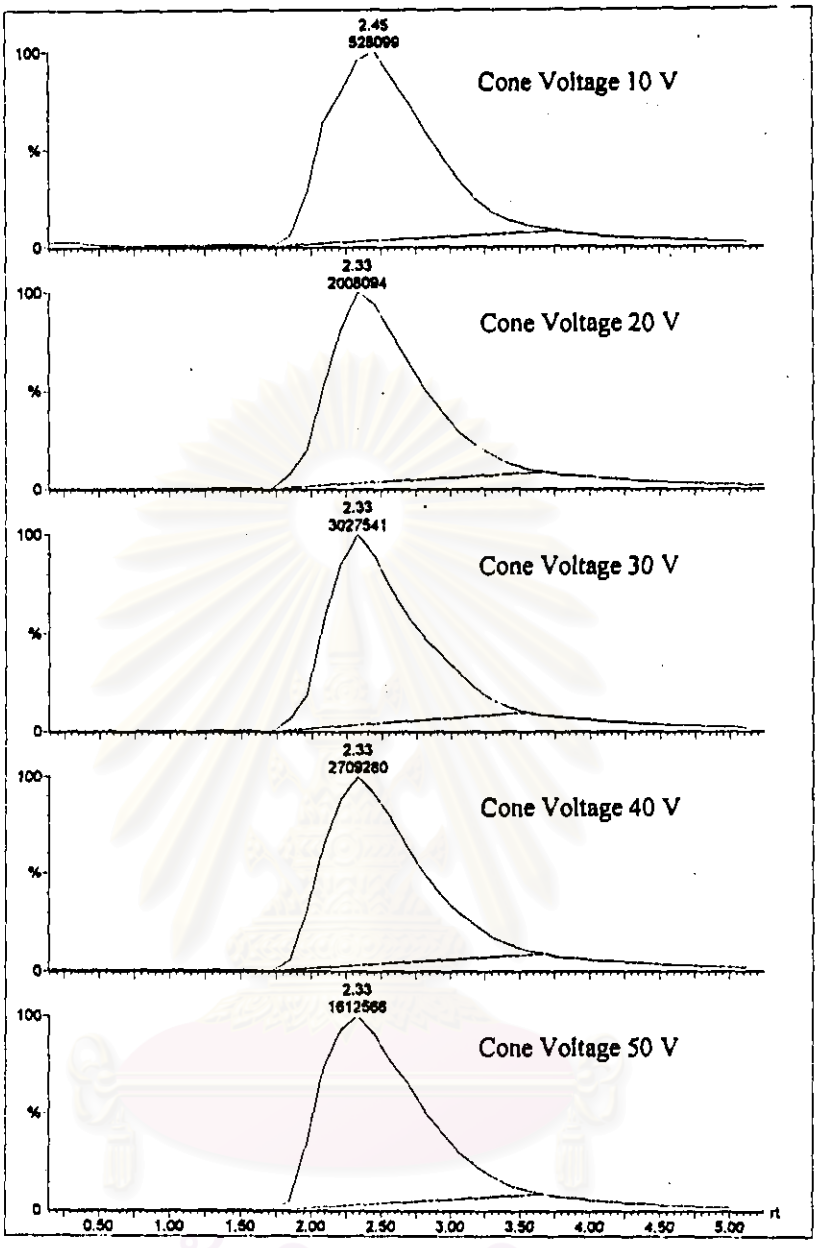


Figure 3.17 SIR signal (at M/Z = 295) of 100 ppm aspartame at various cone voltages obtained under ESI. All other parameters were as follows:

Flow rate : 0.1 mL/min	Lens 2 voltage : 230 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.20 kV	Source temperature : 60 ^{0}C

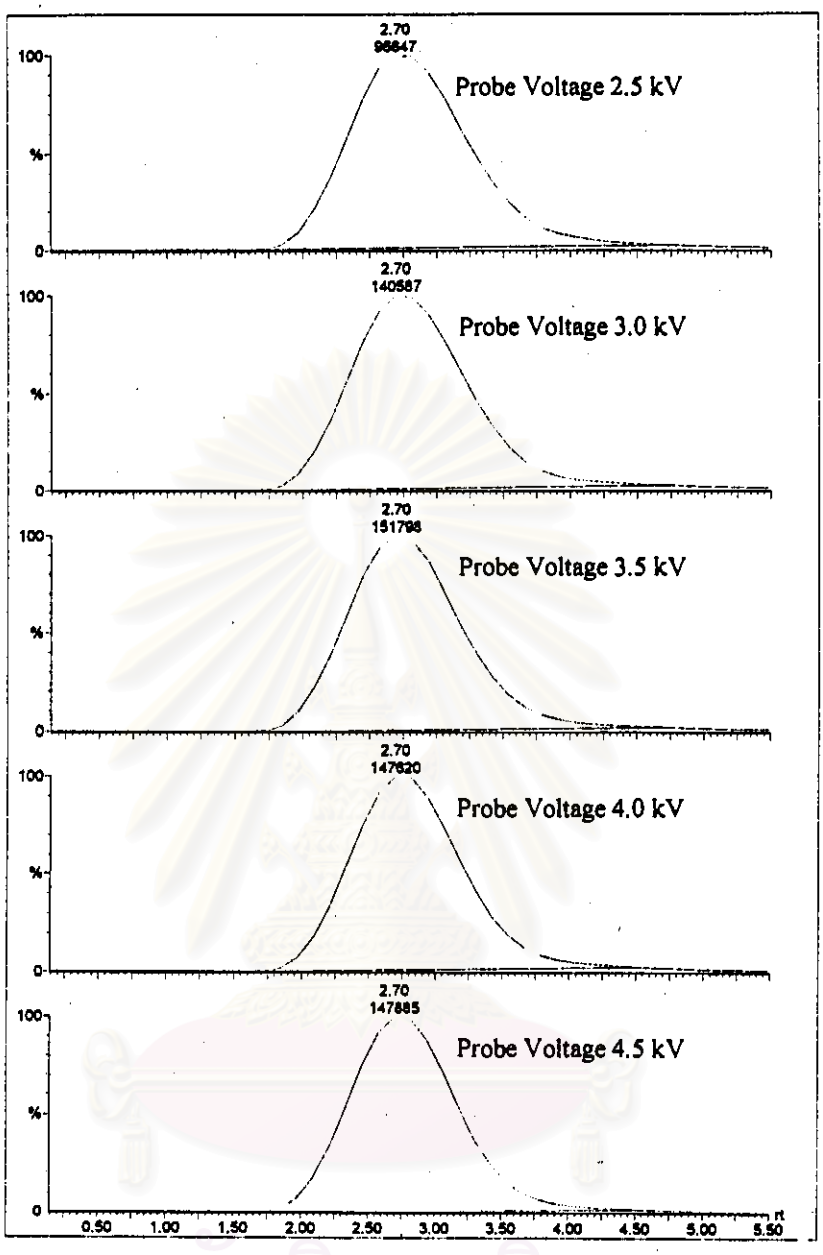


Figure 3.18 SIR signal (at M/Z = 295) of 100 ppm aspartame at various capillary tip voltages obtained under ESI. All other parameters were as follows:

Flow rate : 0.1 mL/min	Lens 2 voltage : 230 V
Counter electrode voltage : 0.15 kV	Lens 3 voltage : 10 V
Cone voltage : 30 V	Source temperature : 60 ^{0}C

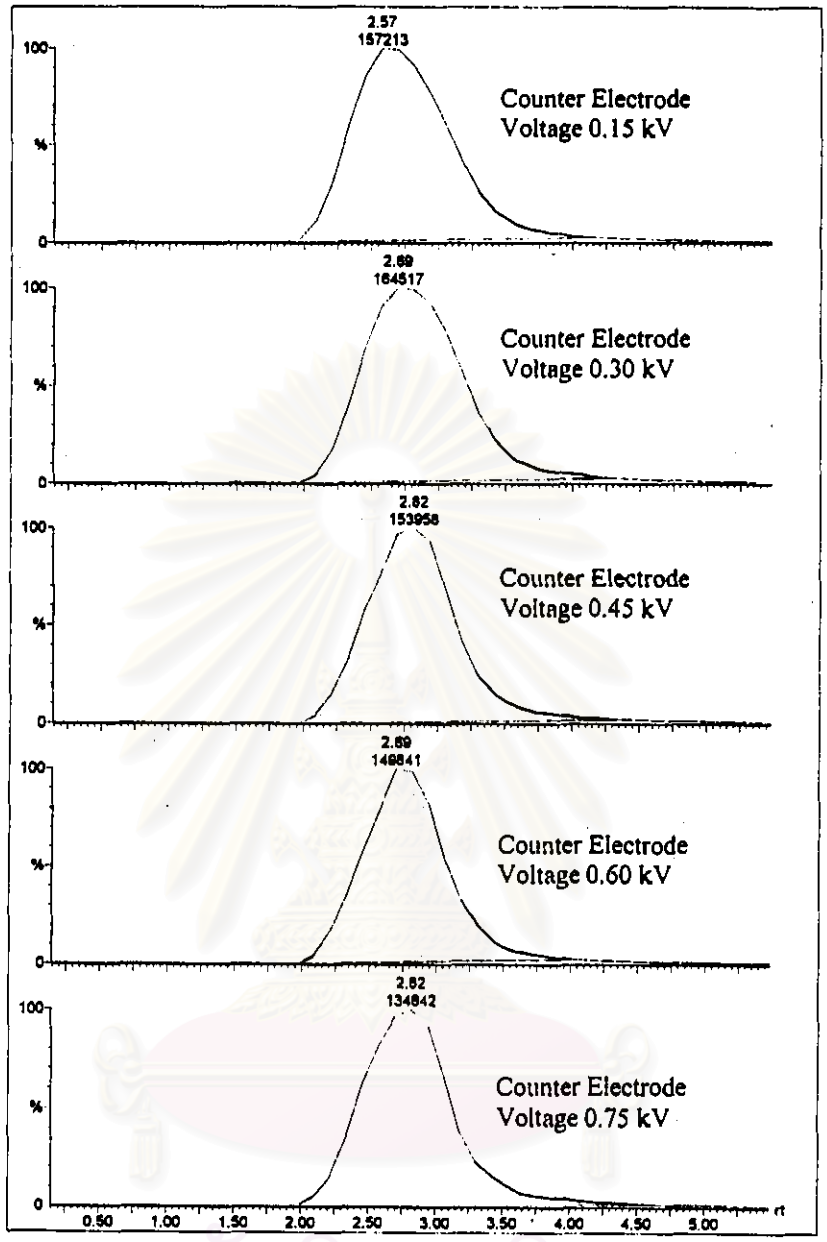


Figure 3.19 SIR signal (at M/Z = 295) of 100 ppm aspartame at various counter electrode voltages obtained under ESI. All other parameters were as follows:

Flow rate : 0.1 mL/min	Lens 2 voltage : 230 V
Capillary tip voltage : 3.50 kV	Lens 3 voltage : 10 V
Cone voltage : 30 V	Source temperature : 60 ^{0}C

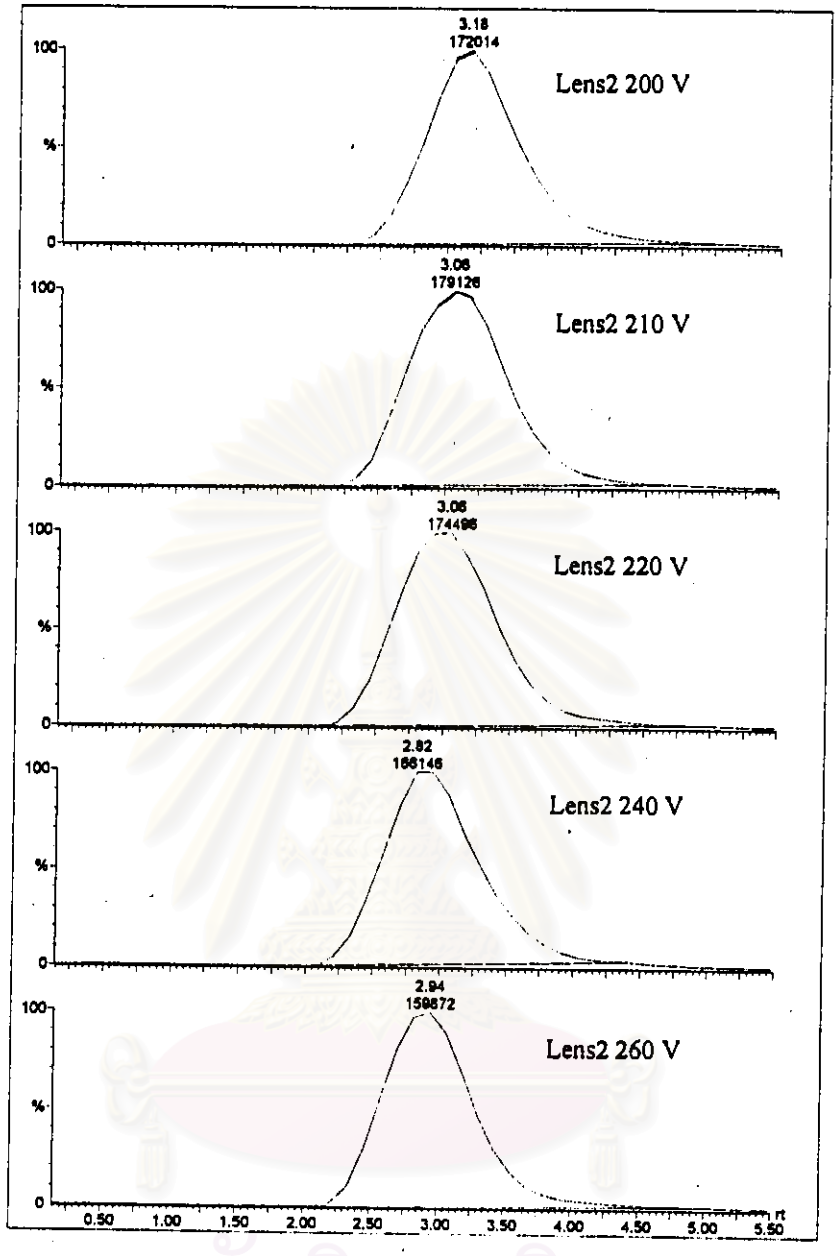


Figure 3.20 SIR signal (at M/Z = 295) of 100 ppm aspartame at various lens 2 voltages obtained under ESI. All other parameters were as follows:

Flow rate : 0.1 mL/min	Cone voltage : 30 V
Capillary tip voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.30 kV	Source temperature : 60 ^{0}C

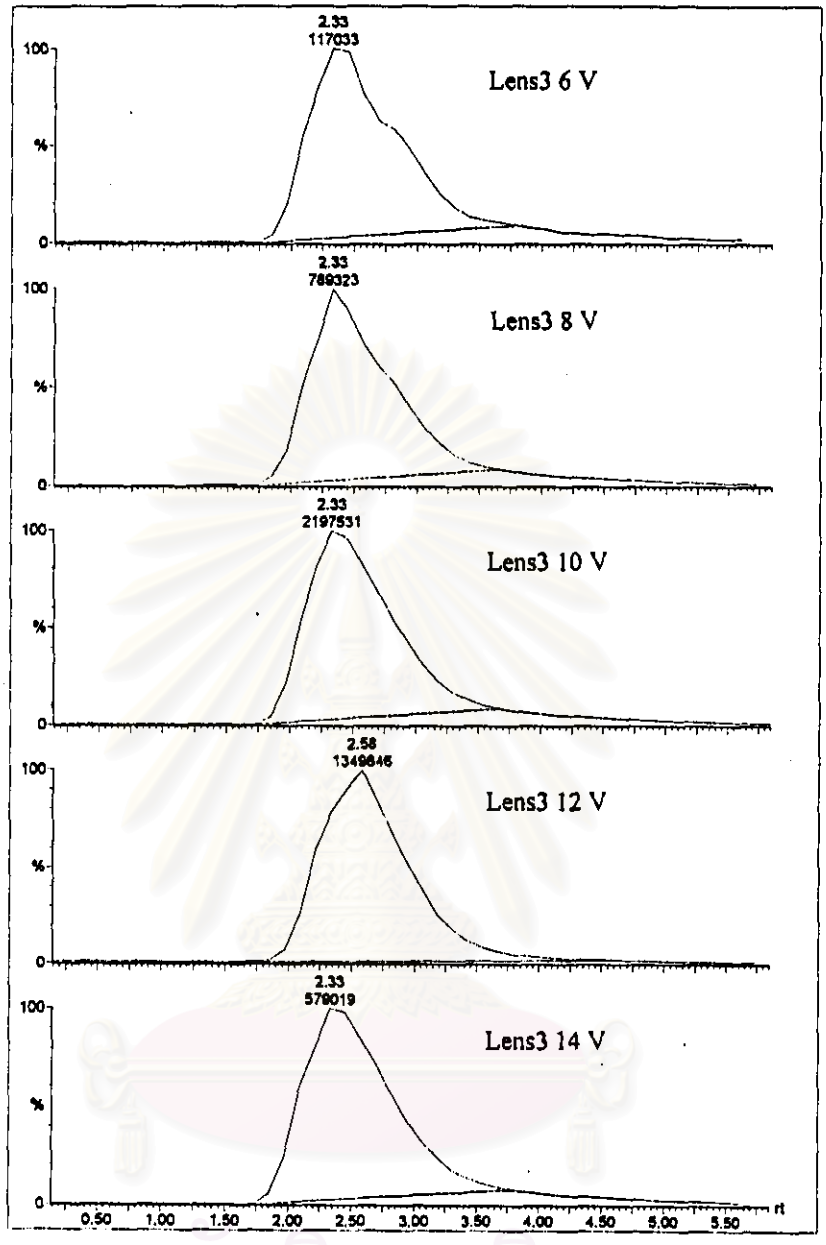


Figure 3.21 SIR signal (at M/Z = 295) of 100 ppm aspartame at various lens 3 voltages obtained under ESI. All other parameters were as follows:

Flow rate : 0.1 mL/min	Cone voltage : 30 V
Capillary tip voltage : 3.50 kV	Lens 2 voltage : 210 V
Counter electrode voltage : 0.30 kV	Source temperature : 60 ^{0}C

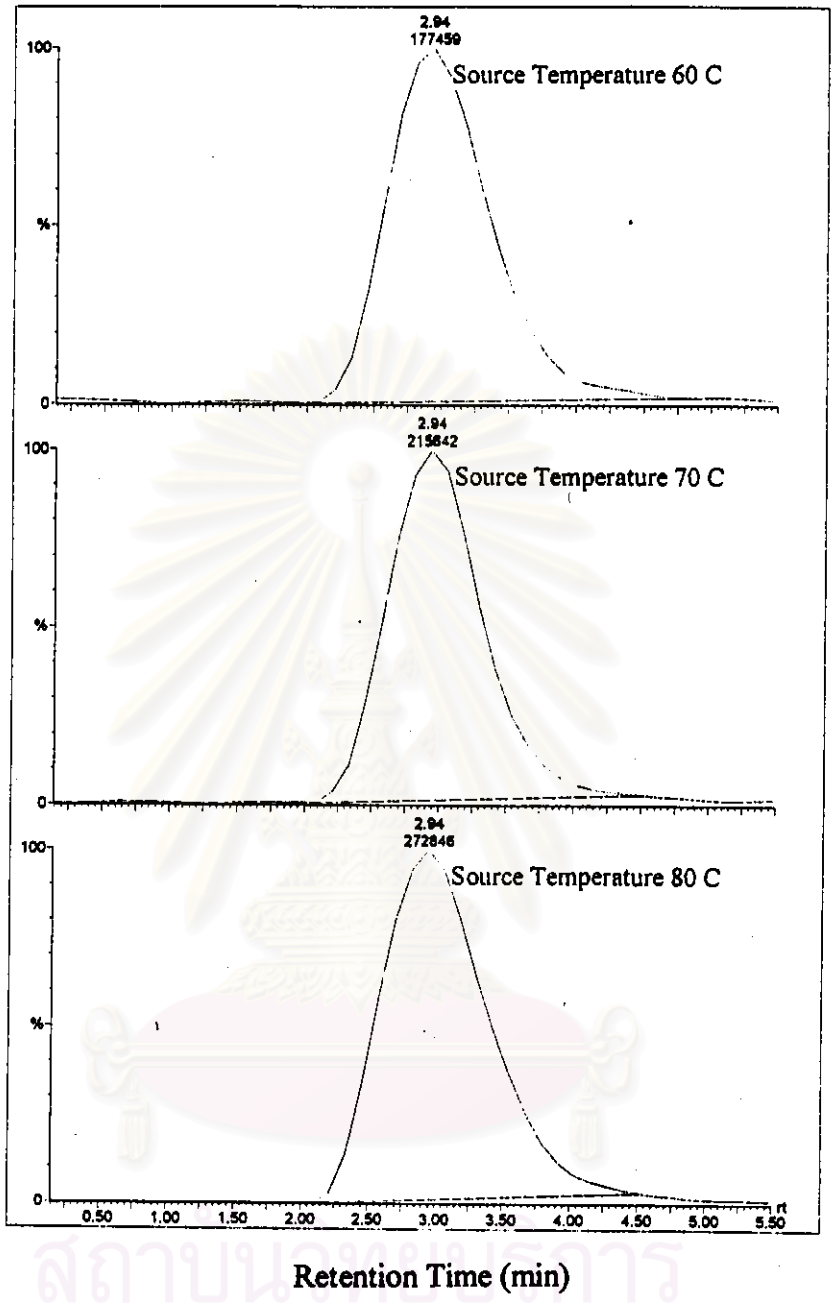


Figure 3.22 SIR signal (at M/Z = 295) of 100 ppm aspartame at various source temperature voltages obtained under ESI. All other parameters were as follows:

Flow rate : 0.1 mL/min	Cone voltage : 30 V
Capillary tip voltage : 3.50 kV	Lens 2 voltage : 210 V
Counter electrode voltage : 0.30 kV	Lens 3 voltage : 10 V

temperature of 80 ^{0}C was, however, used for the rest of experiment because this temperature is the maximum temperature recommended by the manufacture to be safely used with the ESI source.

2.7 **Mobile Phase** Figure 3.23 shows that the size of aspartame molecular peak was not affected by the ratio of acetonitrile:water. The ratio of 20:80 (V/V) acetonitrile:water was used for the rest of the experiment in order to keep the volume of organic solvent at minimum.

2.8 **Flow Rate** Since flow rate of 0.1 mL/min gave stable and prominent aspartame signal together with acceptable time required for each injection, this flow rate was used for the rest of the experiment.

Figure 3.24a shows ESI mass spectrum of 1000 ppm aspartame (50μL injected) obtained under optimal condition, i.e., cone voltage of 30 V, capillary tip voltage of 3.50 kV, counter electrode voltage of 0.20 kV, lens 2 voltage of 210 V, lens 3 voltage of 10 V, source temperature of 80 ^{0}C and flow rate of 0.1 mL/min. Figure 3.24b is the spectrum obtained under the same condition as performed in Figure 3.24a but no aspartame solution was injected, only mobile phase was passed into the ionization source. By comparing Figure 3.24a and 3.24b, it is obvious that peaks at M/Z of 42, 60, 83, 101, and 119 in aspartame spectrum belong to the the mobile phase. These peaks represent cluster ions of $(CH_3CN)H^+$, $(CH_3CN{\bullet}H_2O)H^+$, $(2CH_3CN)H^+$, $(2CH_3CN{\bullet}H_2O)H^+$ and $(2CH_3CN{\bullet}2H_2O)H^+$, respectively. It is very clear that at optimal condition, ESI spectrum of aspartame reveals negligible fragmentation of aspartame molecular ions (Figure 3.24a). Peaks at M/Z of 336 and 227 are negligible (in term of their intensities). Structures of these two ions are shown

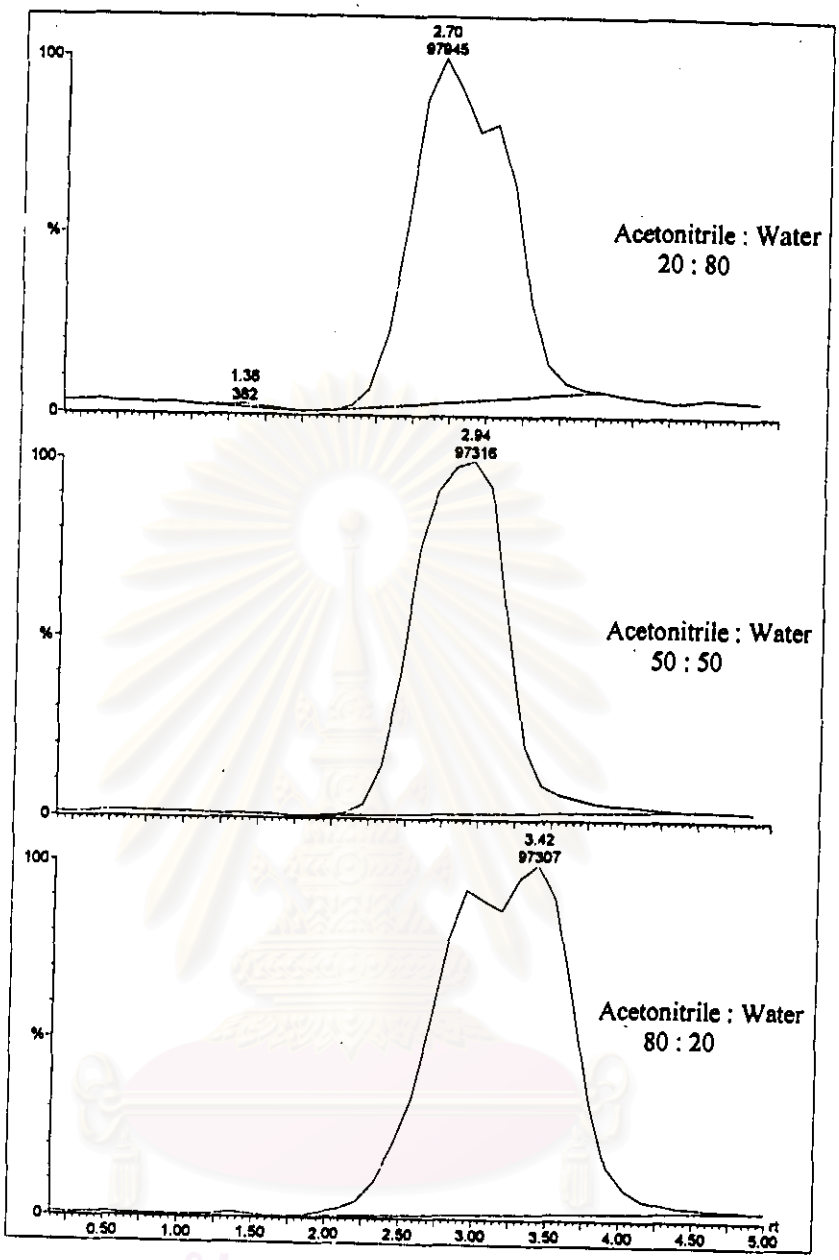


Figure 3.23 SIR signal (at M/Z = 295) of 100 ppm aspartame obtained under various mobile phase components. All other parameters were as follows:

Corona discharge pin voltage : 3.50 kV	Lens 2 voltage : 210 V
Counter electrode voltage : 0.30 kV	Lens 3 voltage : 10 V
Cone voltage : 30 V	Source temperature : 80 ^{0}C

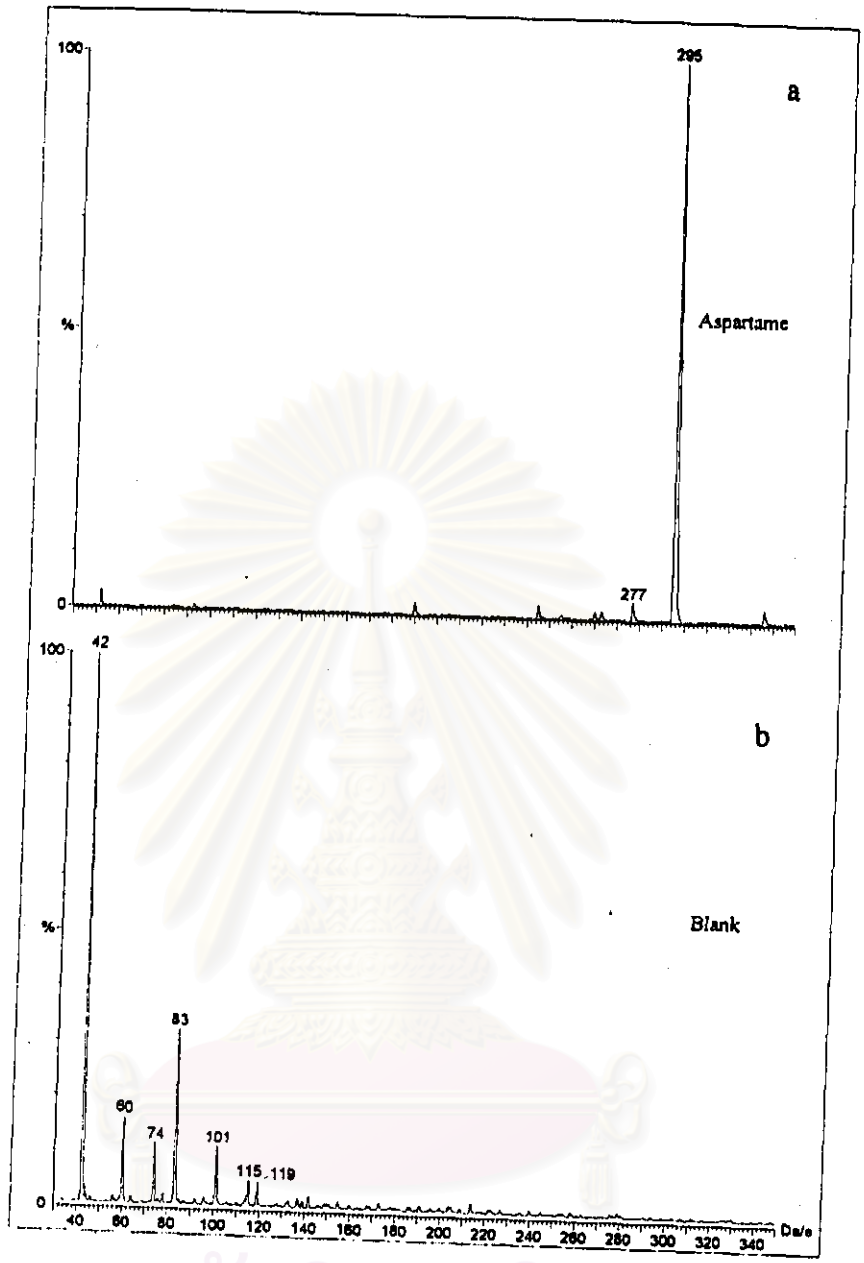


Figure 3.24 ESI mass spectrum of a) 1000 ppm aspartame and b) mobile phase under optimal condition.

Flow rate : 0.1 mL/min

Corona discharge pin voltage : 3.50 kV

Counter electrode voltage : 0.30 kV

Cone voltage : 30 V

Lens 2 voltage : 210 V

Lens 3 voltage : 10 V

Source temperature : 80 ^{0}C

in Table 3.8. Mass spectrum of aspartame degradation products including aspartic acid, aspartyl phenylalanine, diketopiperazine, methanol, phenylalanine and phenylalanine methyl ester, are shown in Figure 3.25 - 3.30, respectively. Table 3.8 - 3.11 show structures of ions representing peaks in ESI spectrum of aspartame, aspartic acid, aspartyl phenylalanine and phenylalanine, respectively. From these results, it is clear that at optimal condition of ESI, all compounds representing aspartame degradation products did not fragment during ionization. Protonated molecular peak was the base peak in all spectra (excluding solvent peak at M/Z of 42 in the case of methanol).

Table 3.8 Some fragment ions from the ionization of aspartame by ESI.

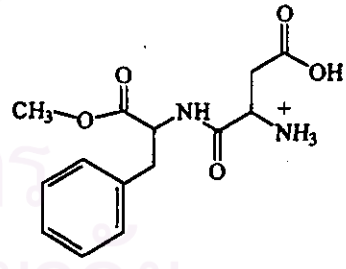
M/Z	Ions	Fragments
336	(M+1)+41	Protonated Aspartame + CH_3CN
295	M+1	 Protonated Aspartame

Table 3.8 (continue)

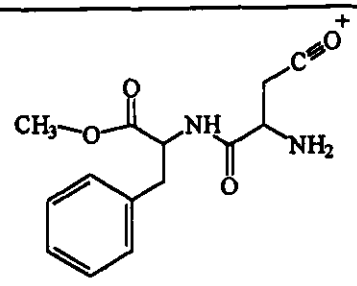
M/Z	Ions	Fragments
277	(M+1)-18	

Table 3.9 Some fragment ions from the ionization of aspartic acid by ESI.

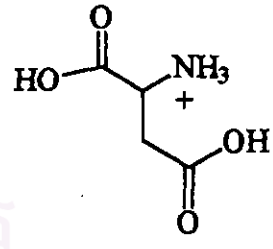
M/Z	Ions	Fragments
216	(M+1)+82	Protonated Aspartic acid + 2 CH_3CN
175	(M+1)+41	Protonated Aspartic acid + CH_3CN
134	M+1	 Protonated Aspartic acid

Table 3.10 Some fragment ions from the ionization of aspartyl phenylalanine by ESI.

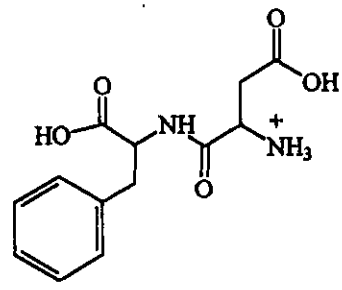
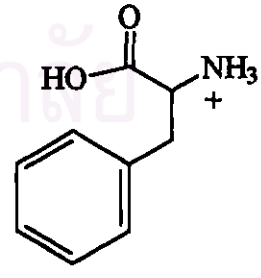
M/Z	Ions	Fragments
322	(M+1)+41	Protonated Aspartyl phenylalanine $+CH_3CN$
281	M+1	 Protonated Aspartyl phenylalanine

Table 3.11 Some fragment ions from the ionization of phenylalanine by ESI.

M/Z	Ions	Fragments
207	(M+1)+41	Protonated Phenylalanine $+ CH_3CN$
166	M+1	 Protonated Phenylalanine

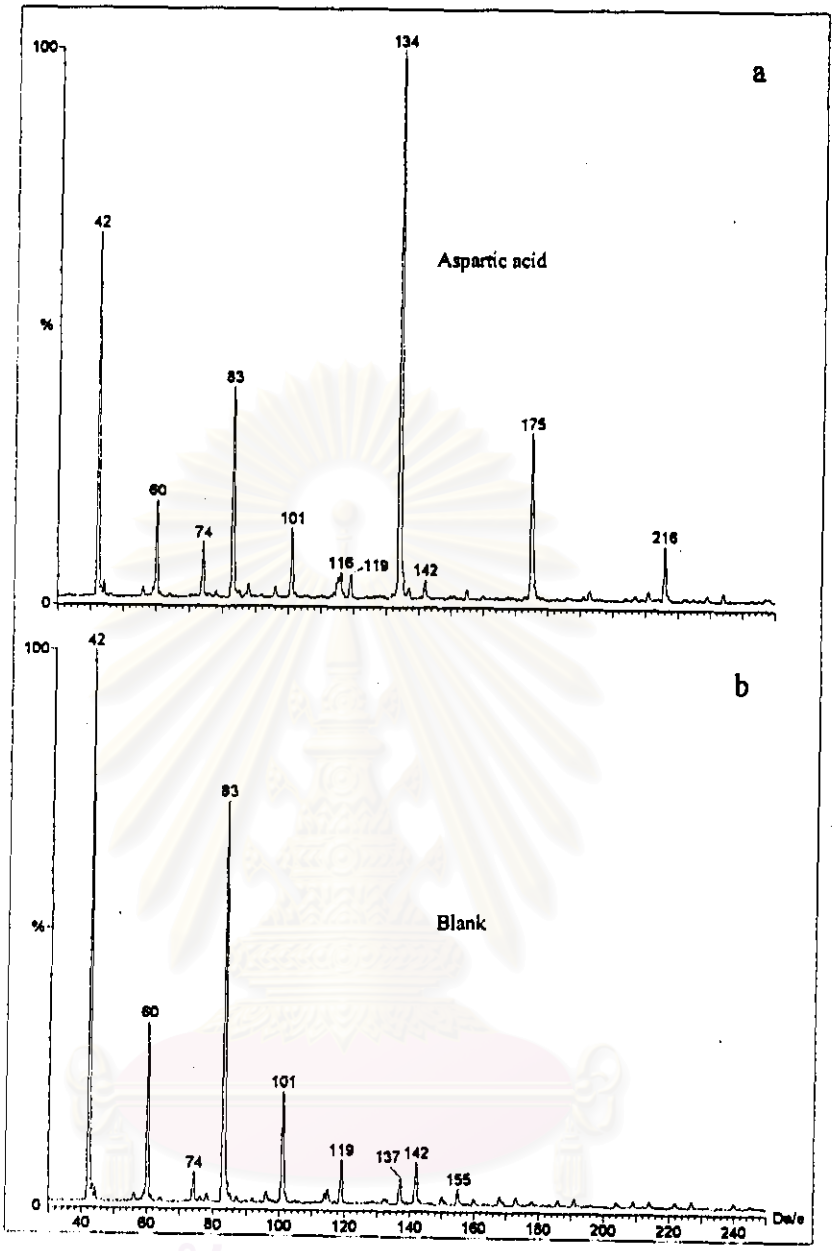


Figure 3.25 ESI mass spectrum of a) 1000 ppm aspartic acid and b) mobile phase under optimal condition.

Flow rate : 0.1 mL/min	Lens 2 voltage : 210 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.30 kV	Source temperature : 80 ^{0}C
Cone voltage : 30 V	

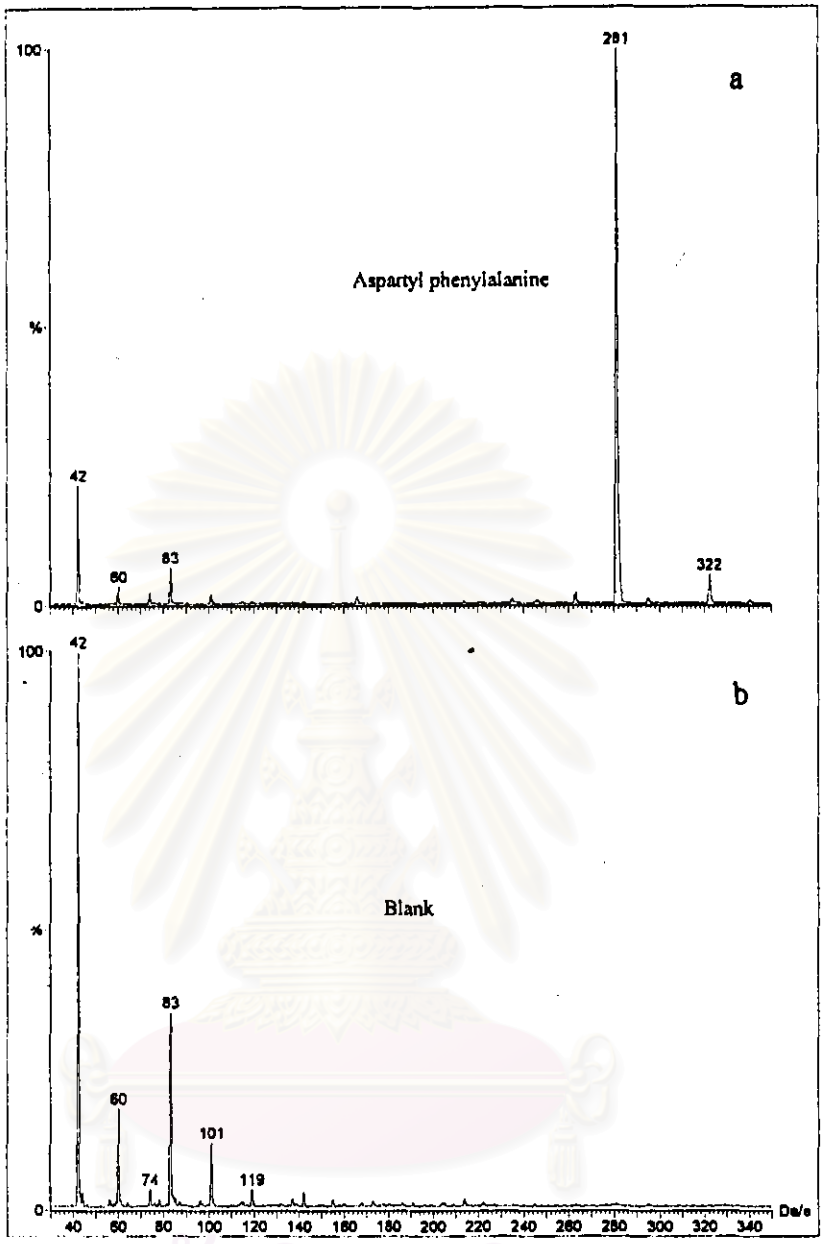


Figure 3.26 ESI mass spectrum of a) 200 ppm aspartyl phenylalanine and b) mobile phase under optimal condition.

Flow rate : 0.1 mL/min	Lens 2 voltage : 210 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.30 kV	Source temperature : 80 ^{0}C
Cone voltage : 30 V	

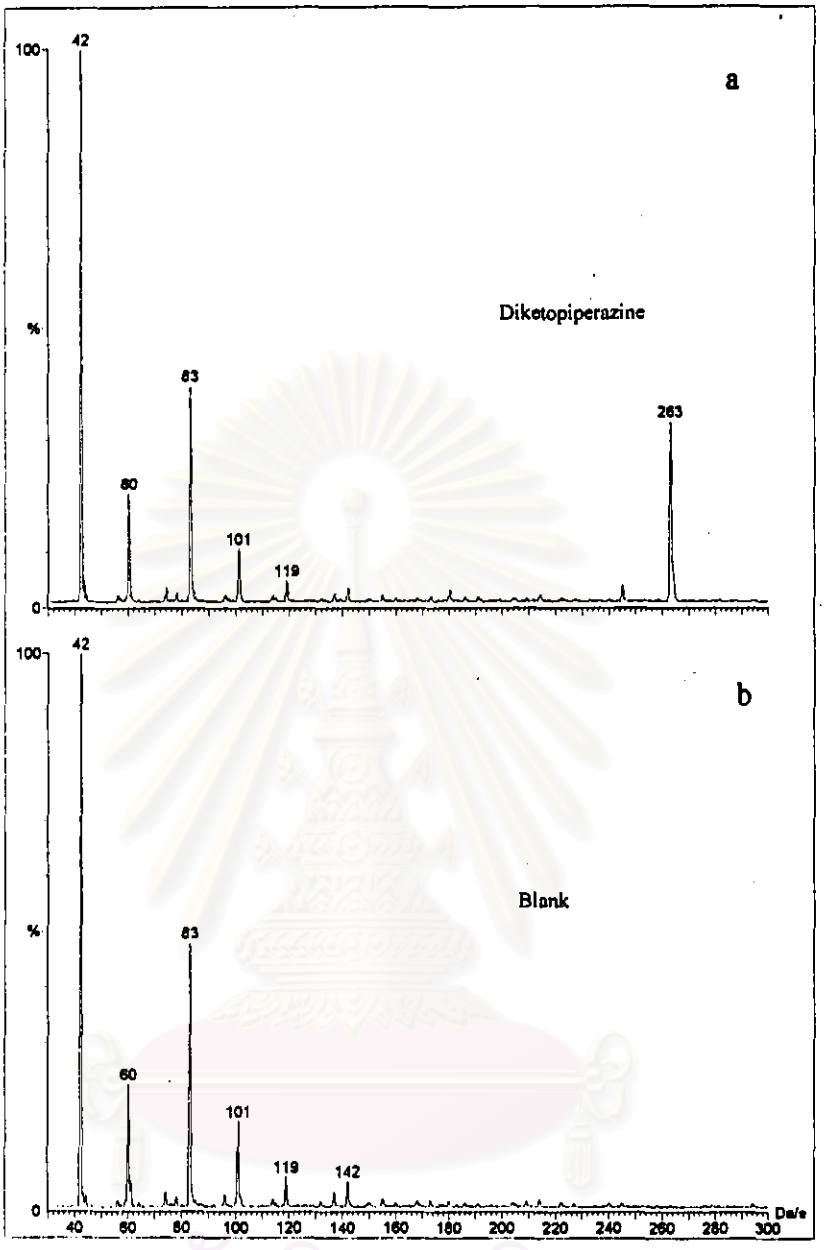


Figure 3.27 ESI mass spectrum of a) 150 ppm diketopiperazine and b) mobile phase under optimal condition.

Flow rate : 0.1 mL/min	Lens 2 voltage : 210 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.30 kV	Source temperature : 80 ^{0}C
Cone voltage : 30 V	

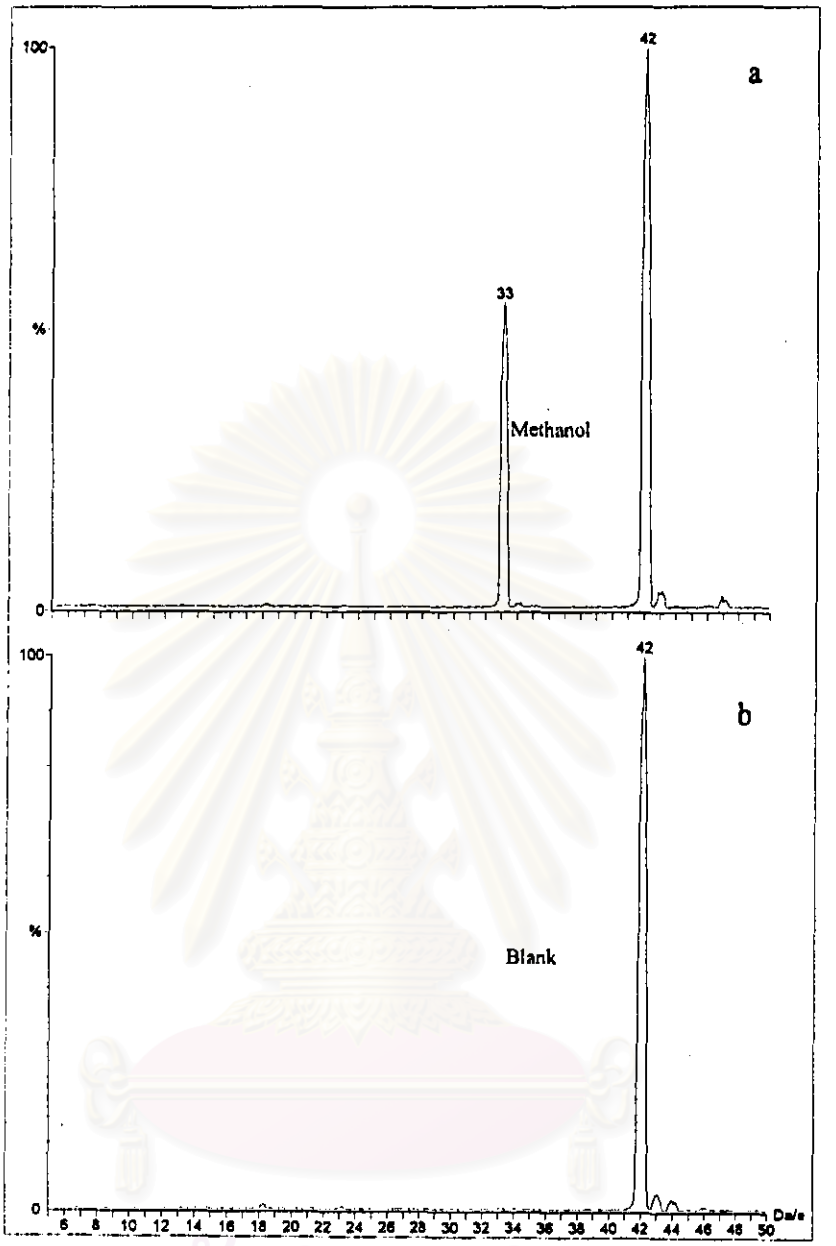


Figure 3.28 ESI mass spectrum of a) Absolute methanol and b) mobile phase under optimal condition.

Flow rate : 0.1 mL/min	Lens 2 voltage : 210 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.30 kV	Source temperature : 80 ^{0}C
Cone voltage : 30 V	

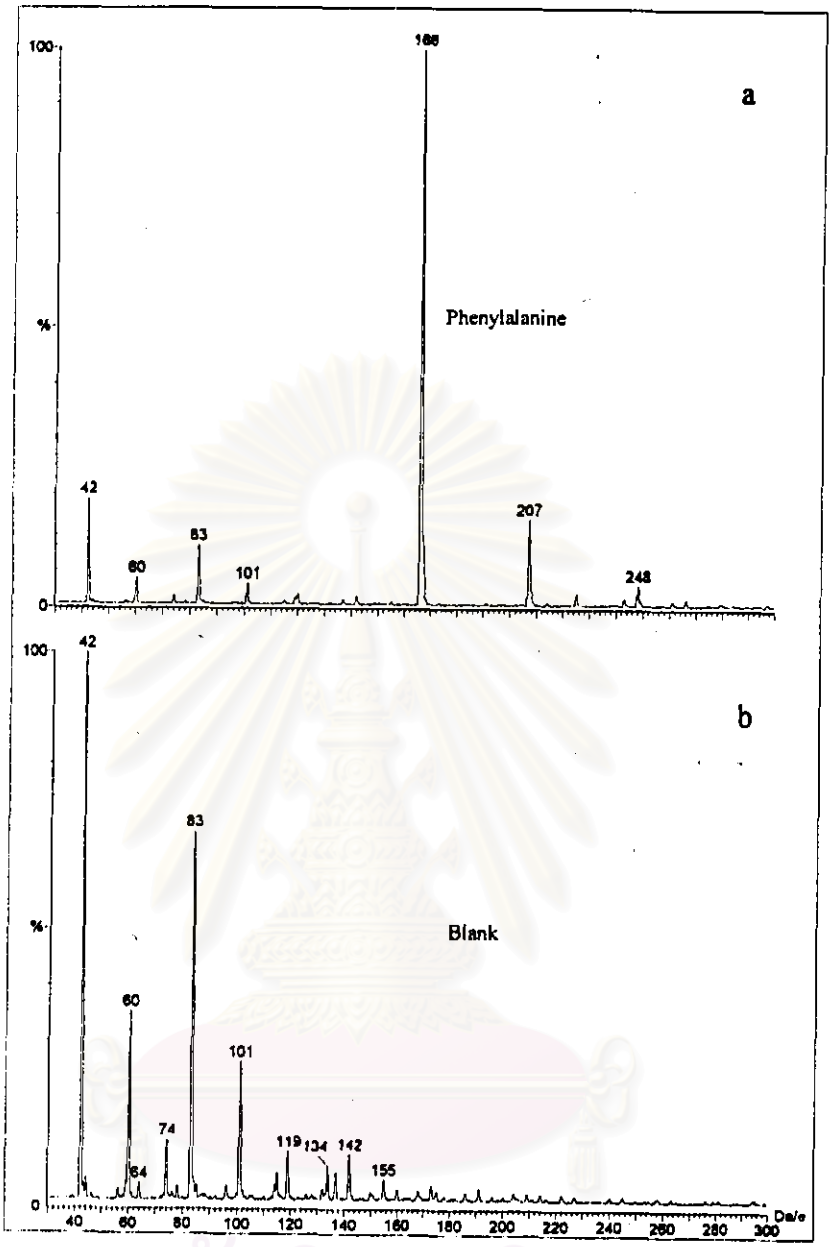


Figure 3.29 ESI mass spectrum of a) 1000 ppm phenylalanine and b) mobile phase under optimal condition.

Flow rate : 0.1 mL/min	Lens 2 voltage : 210 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.30 kV	Source temperature : 80 ^{0}C
Cone voltage : 30 V	

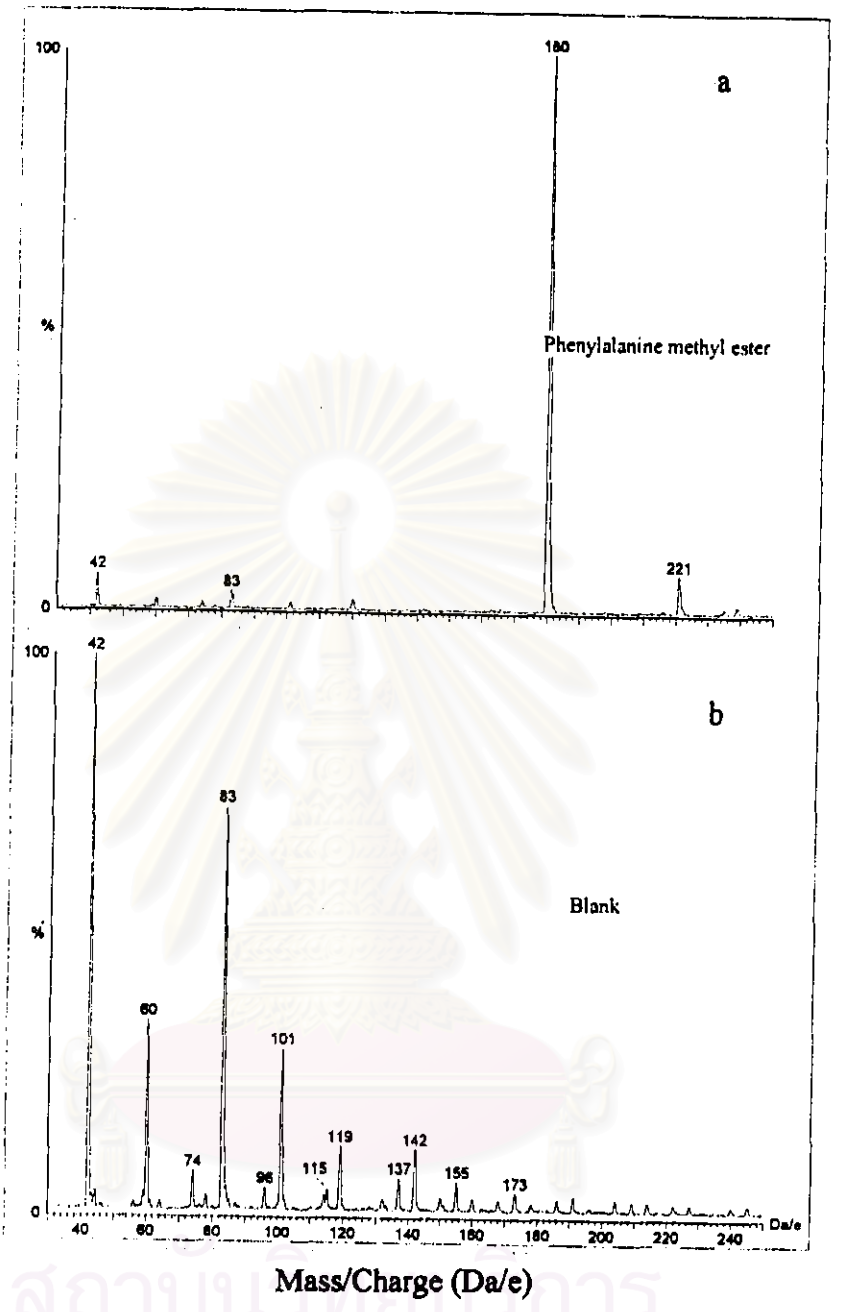


Figure 3.30 ESI mass spectrum of a) 1000 ppm phenylalanine methyl ester and b) mobile phase under optimal condition.

Flow rate : 0.1 mL/min	Lens 2 voltage : 210 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.30 kV	Source temperature : 80 ^{0}C
Cone voltage : 30 V	

3. **Detection Limit Experiment**

Since the goal of this study is the study of aspartame stability and its degradation chemistry, therefore, not only aspartame was the substance to be monitored, all aspartame degradation products which include aspartic acid, aspartyl phenylalanine, diketopiperazine, methanol, phenylalanine and phenylalanine methyl ester were also needed to be monitored. The best analytical method must, therefore, provide windows to detect all these substances.

In this study, both ESI and APCI were used to detect aspartame, aspartic acid, aspartyl phenylalanine, diketopiperazine, methanol, phenylalanine and phenylalanine methyl ester simultaneously at various pH. In this case the detection limit was the lowest concentration that yielded ratio of the peak height of the analytes to baseline (or noise) ~3. The results are shown in Figure 3.31 - 3.44 and summarized in Table 3.12 and 3.13.

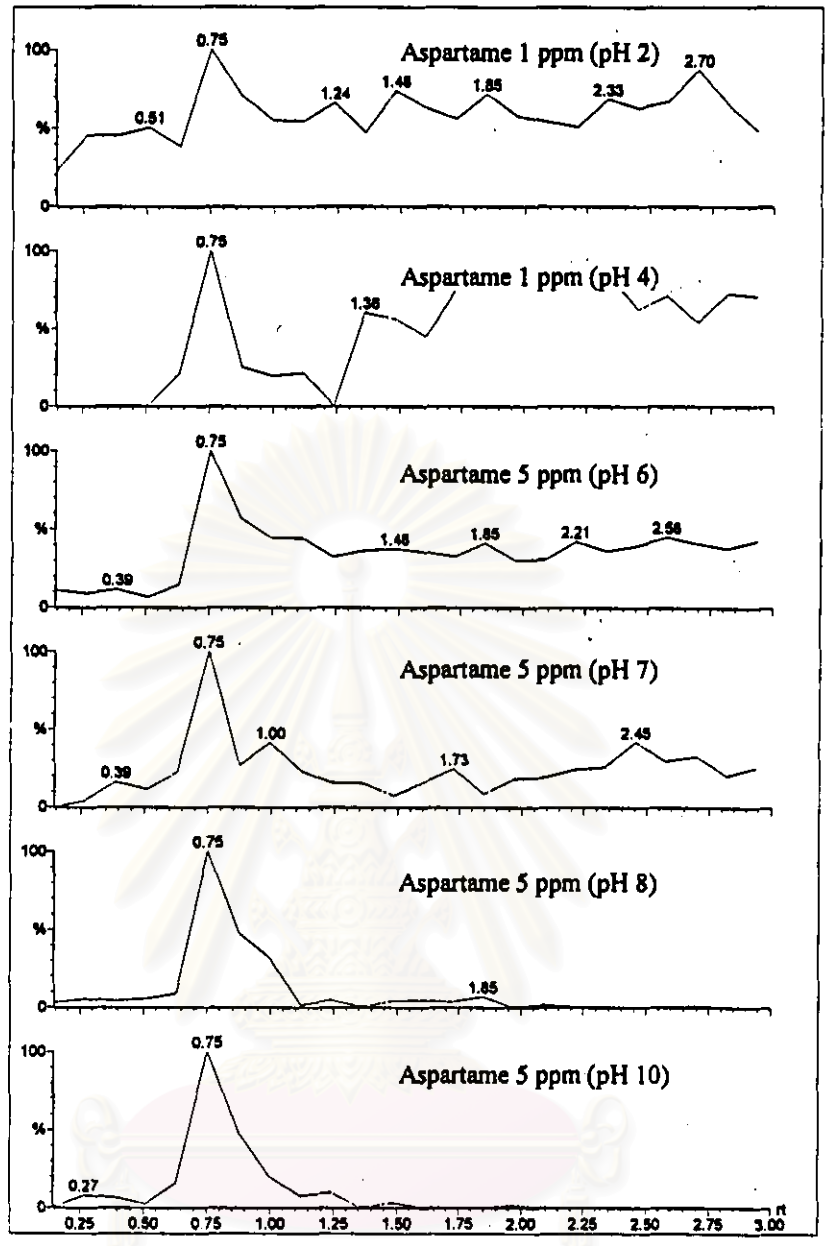


Figure 3.31 APCI SIR signal of 50 μL aspartame solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

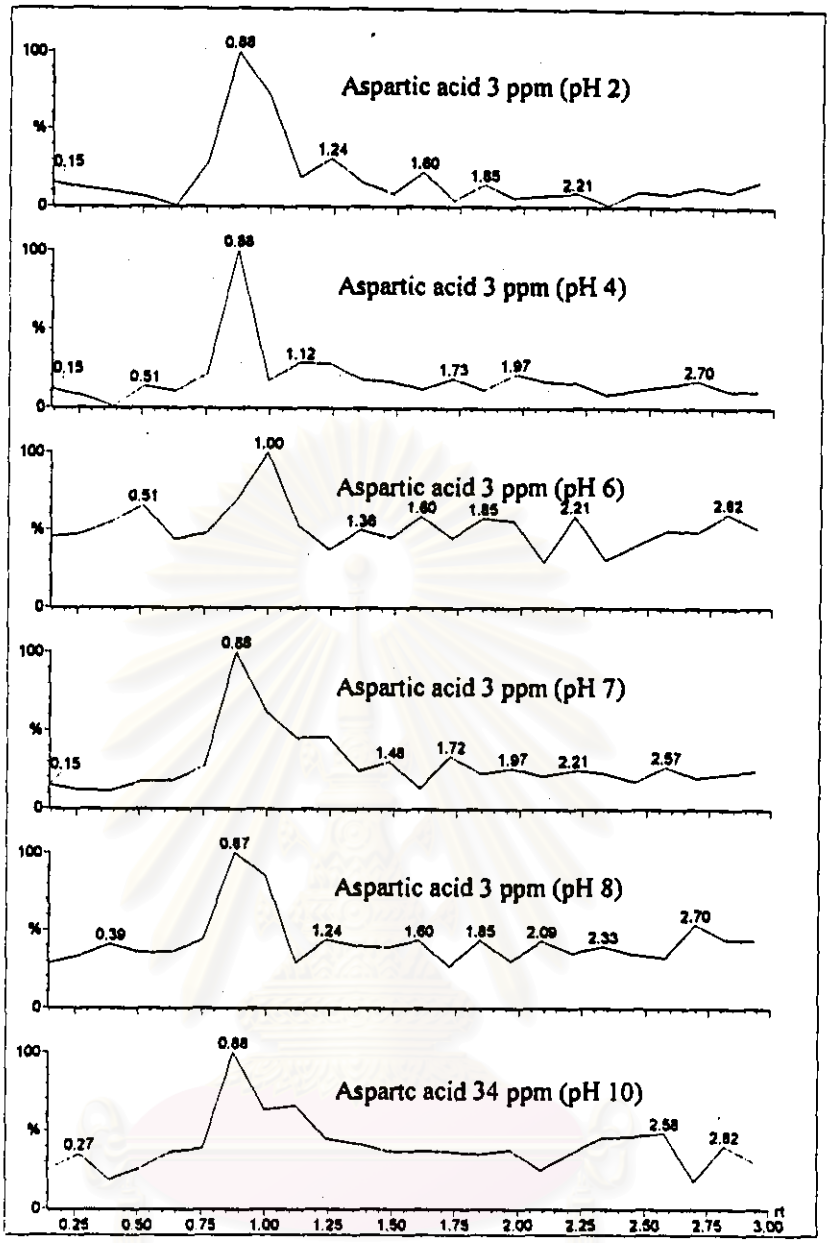


Figure 3.32 APCI SIR signal of 50 μL aspartic acid solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.5 mL/min

Corona discharge pin voltage : 3.50 kV

Counter electrode voltage : 0.40 kV

Cone voltage : 60 V

Lens 2 voltage : 240 V

Lens 3 voltage : 10 V

Source temperature : 120 ^{0}C

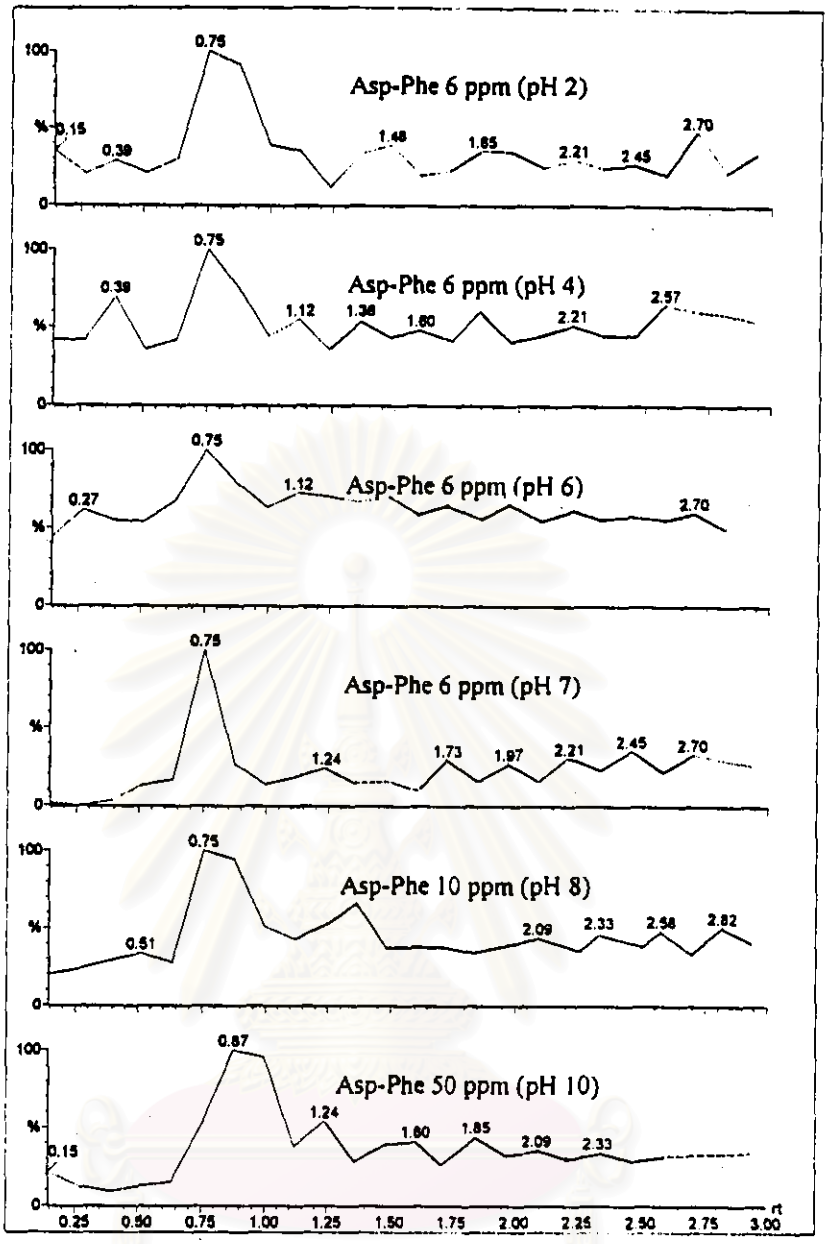


Figure 3.33 APCI SIR signal of 50 μL aspartyl phenylalanine solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 °C
Cone voltage : 60 V	

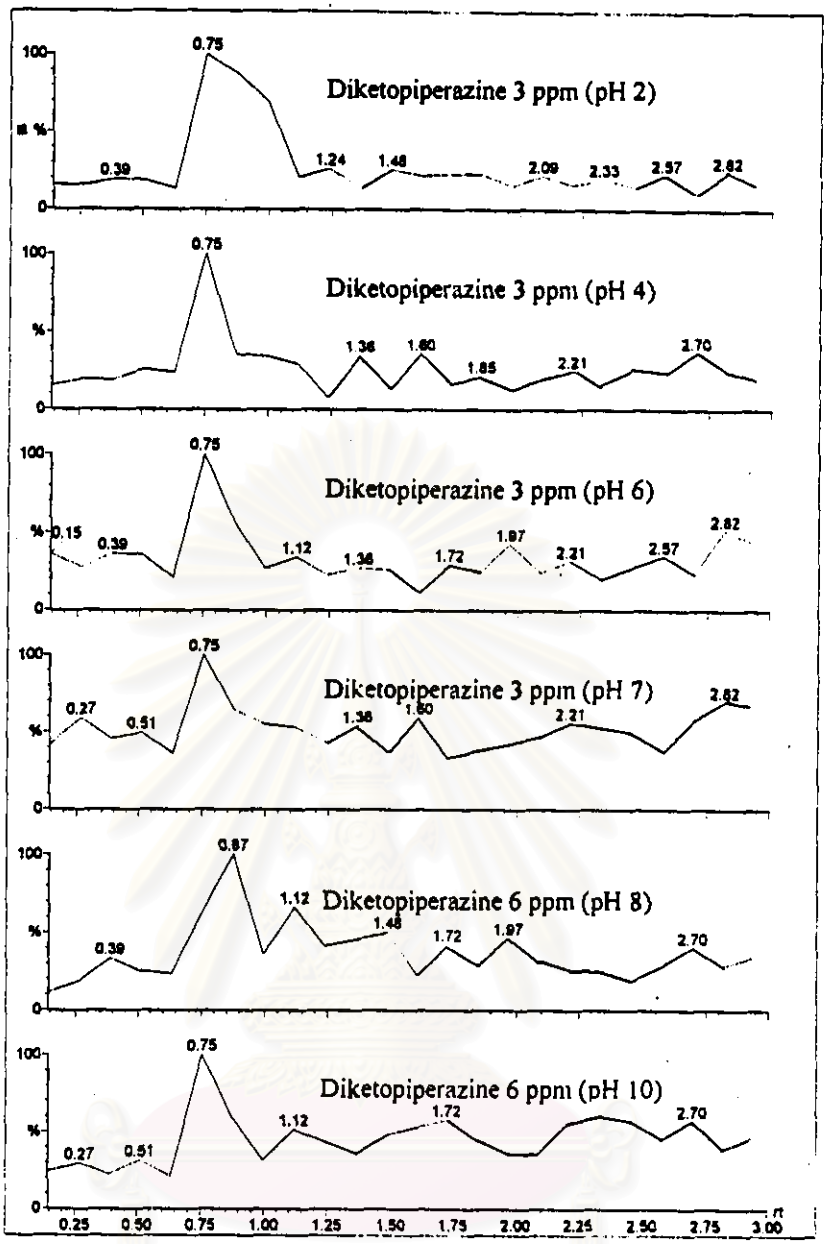


Figure 3.34 APCI SIR signal of 50 µL diketopiperazine solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

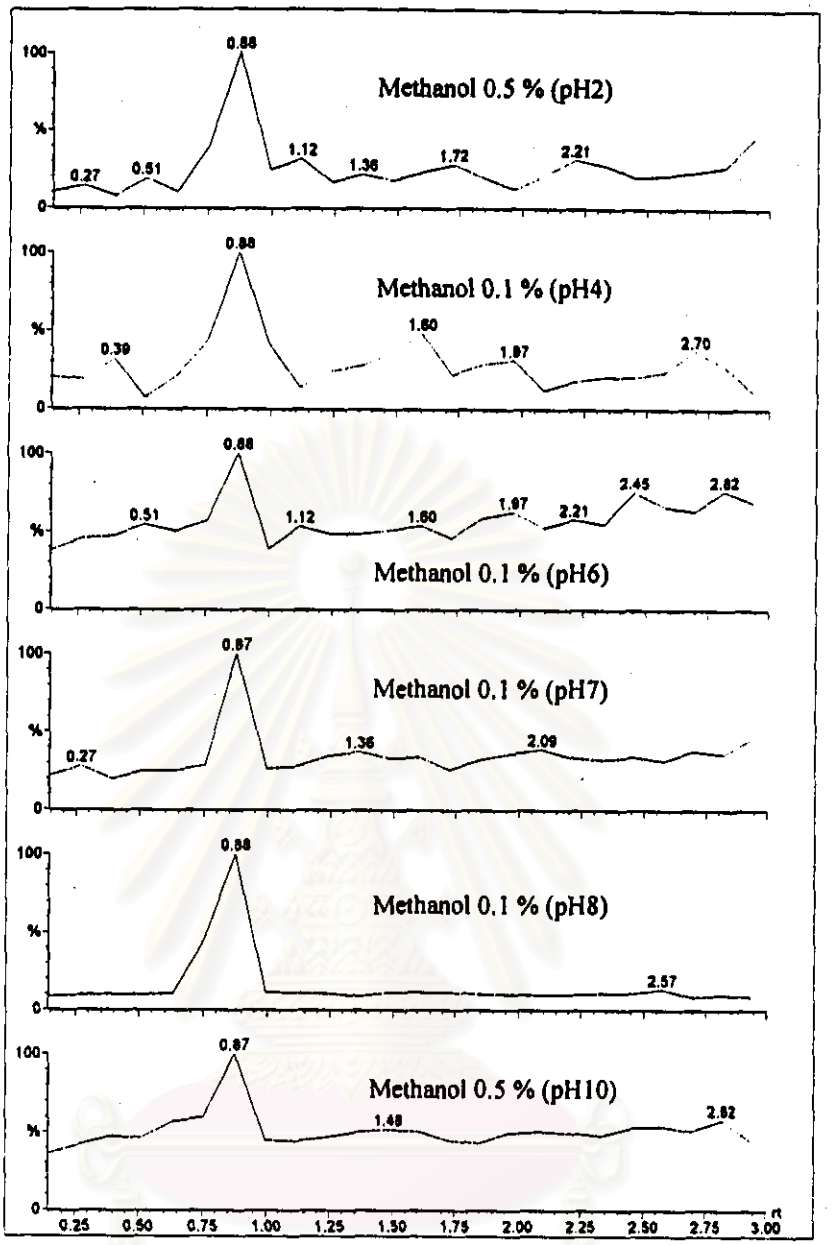


Figure 3.35 APCI SIR signal of 50 µL absolute methanol of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

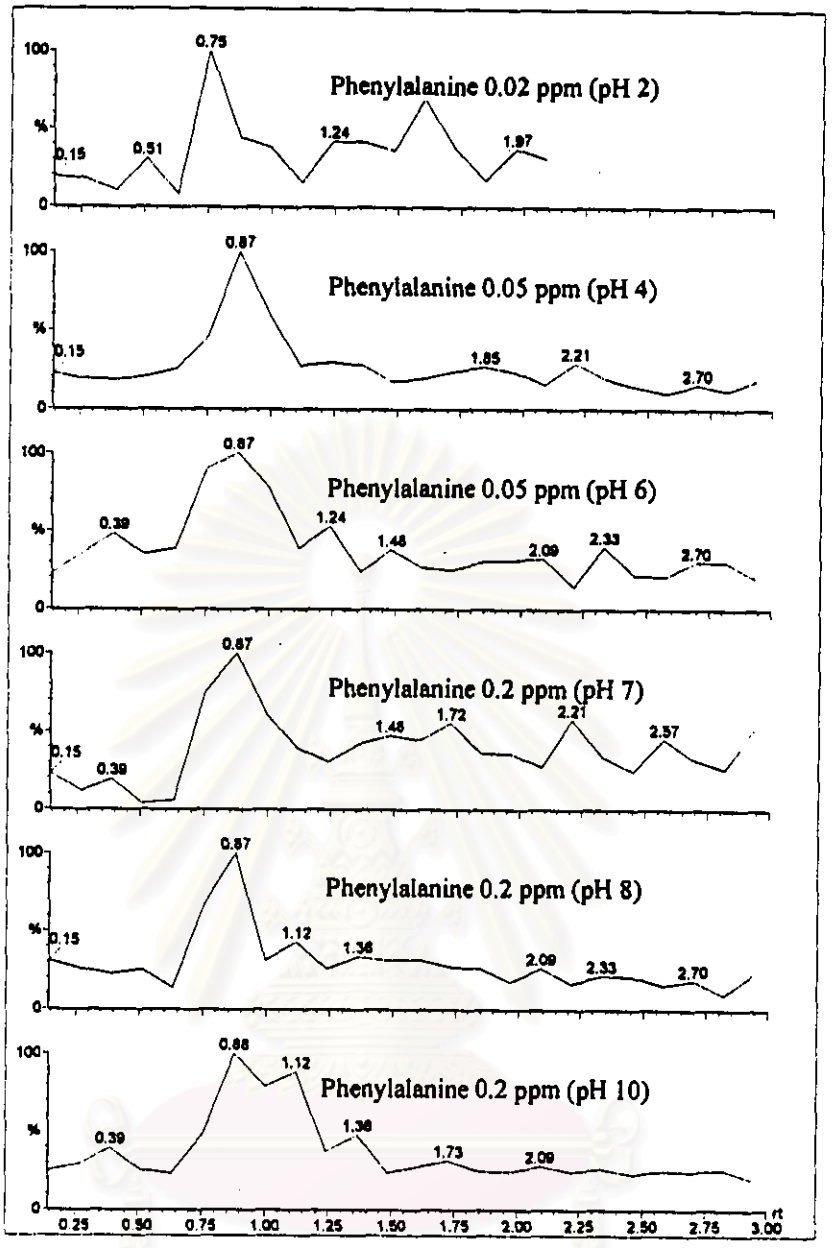


Figure 3.36 APCI SIR signal of 50 μL phenylalanine solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

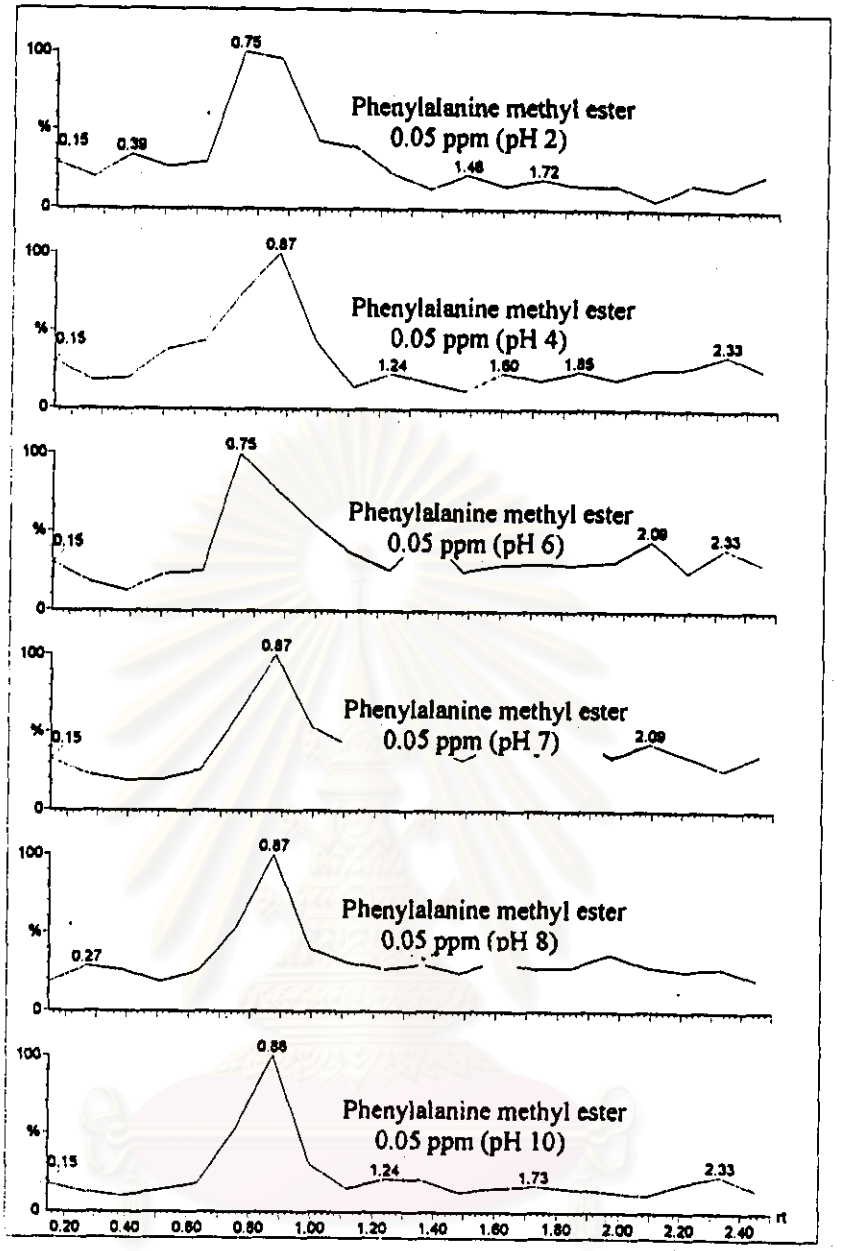


Figure 3.37 APCI SIR signal of 50 μL phenylalanine methyl ester solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.5 mL/min	Lens 2 voltage : 240 V
Corona discharge pin voltage : 3.50 kV	Lens 3 voltage : 10 V
Counter electrode voltage : 0.40 kV	Source temperature : 120 ^{0}C
Cone voltage : 60 V	

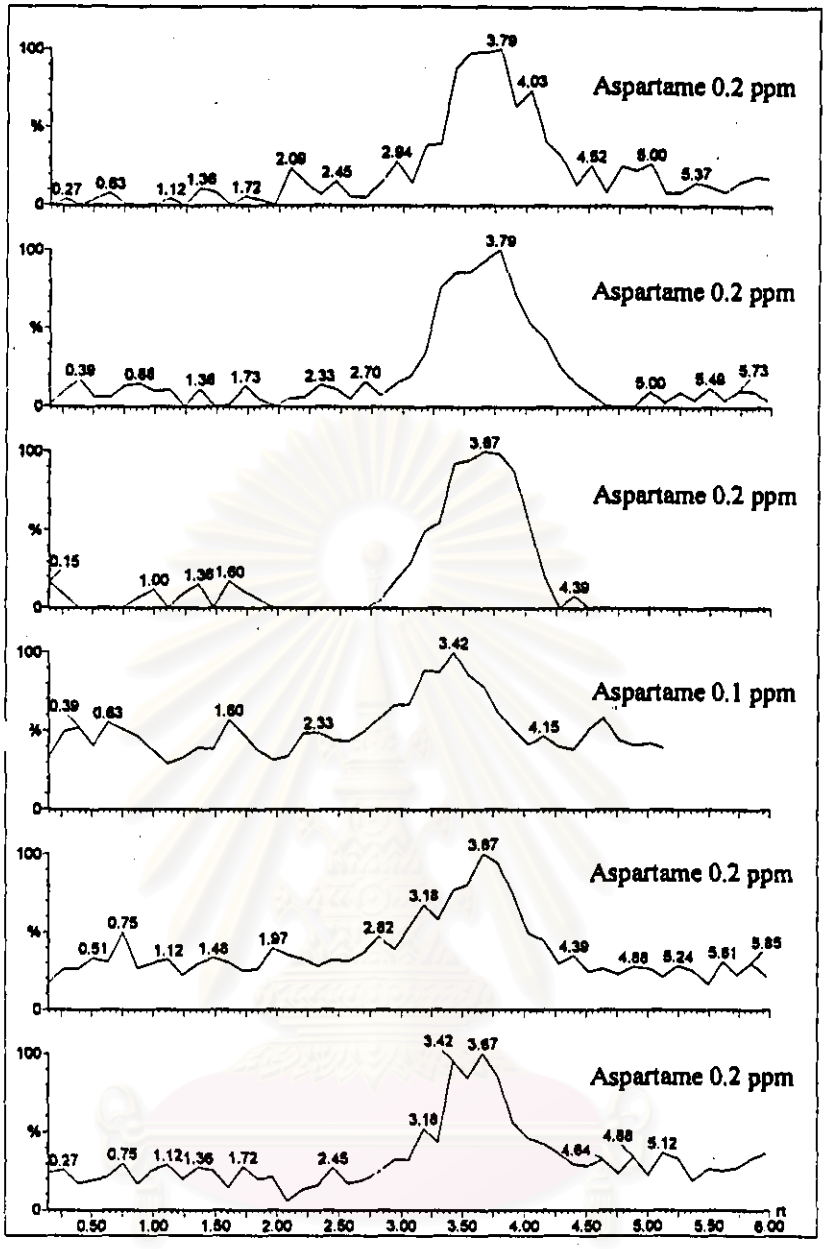


Figure 3.38 ESI SIR signal of 50 μL aspartame solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.1 mL/min Lens 2 voltage : 210 V

Capillary tip voltage : 3.50 kV Lens 3 voltage : 10 V

Counter electrode voltage : 0.30 kV Source temperature : 80 ^{0}C

Cone voltage : 30 V

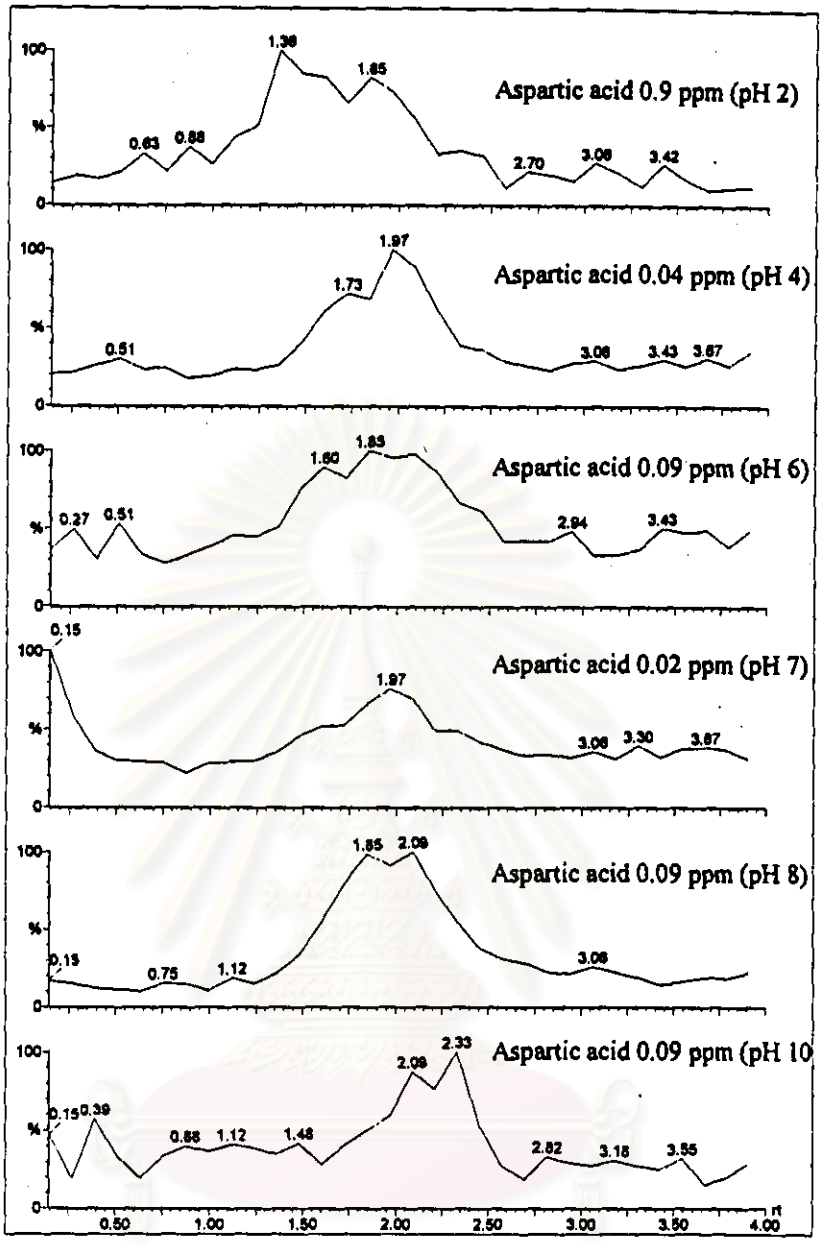


Figure 3.39 ESI SIR signal of 50 µL aspartic acid solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.1 mL/min Lens 2 voltage : 210 V

Capillary tip voltage : 3.50 kV Lens 3 voltage : 10 V

Counter electrode voltage : 0.30 kV Source temperature : 80 ^{0}C

Cone voltage : 30 V

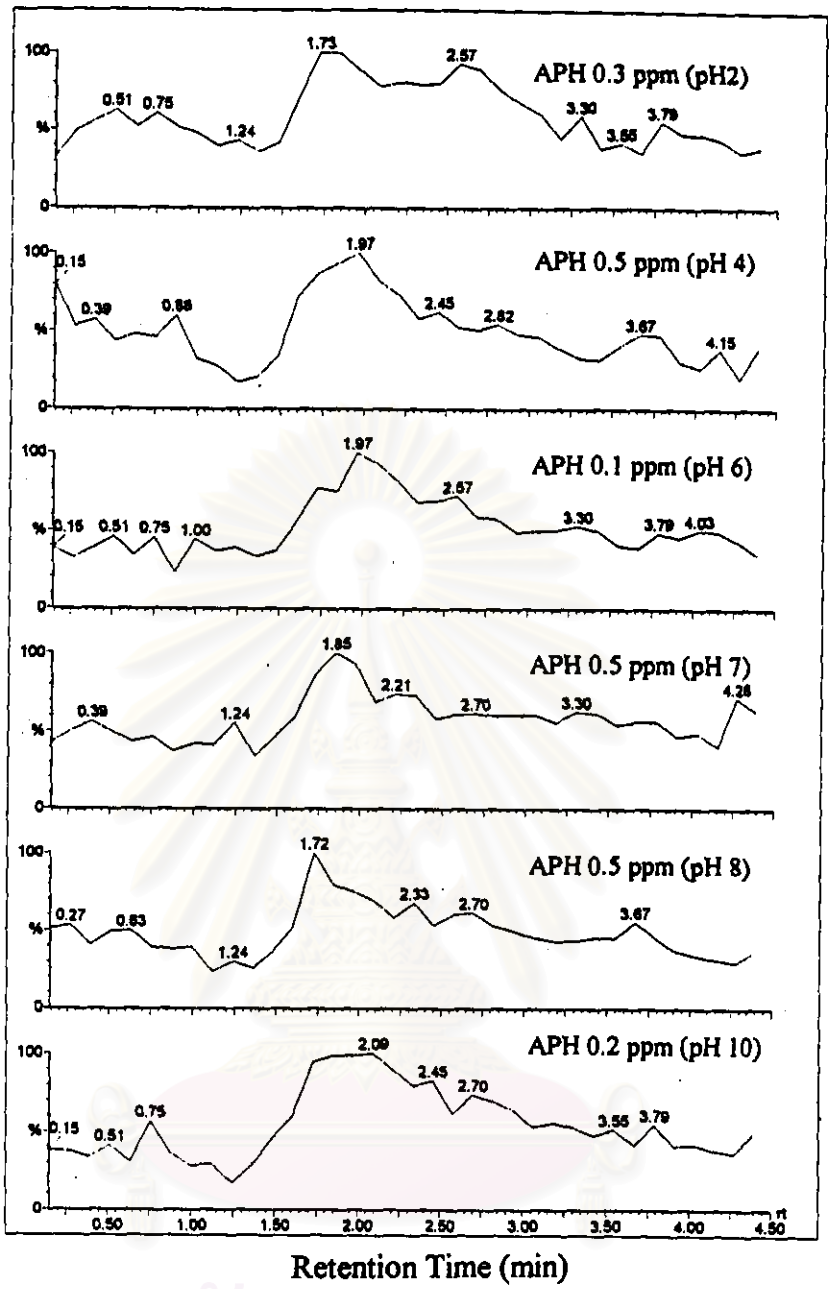


Figure 3.40 ESI SIR signal of 50 μL aspartyl phenylalanine solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.1 mL/min Lens 2 voltage : 210 V

Capillary tip voltage : 3.50 kV Lens 3 voltage : 10 V

Counter electrode voltage : 0.30 kV Source temperature : 80 ^{0}C

Cone voltage : 30 V

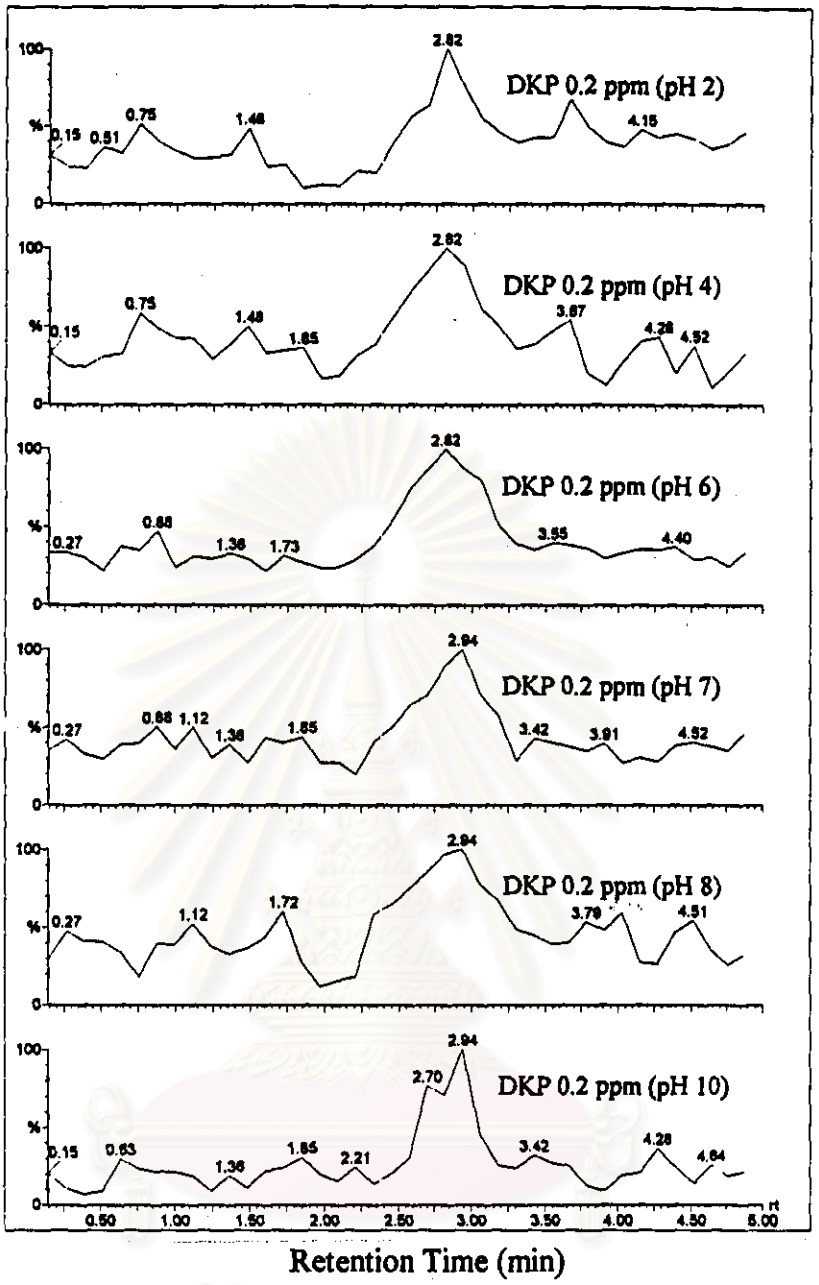


Figure 3.41 ESI SIR signal of 50 μL diketopiperazine solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.1 mL/min Lens 2 voltage : 210 V

Capillary tip voltage : 3.50 kV Lens 3 voltage : 10 V

Counter electrode voltage : 0.30 kV Source temperature : 80 ^{0}C

Cone voltage : 30 V

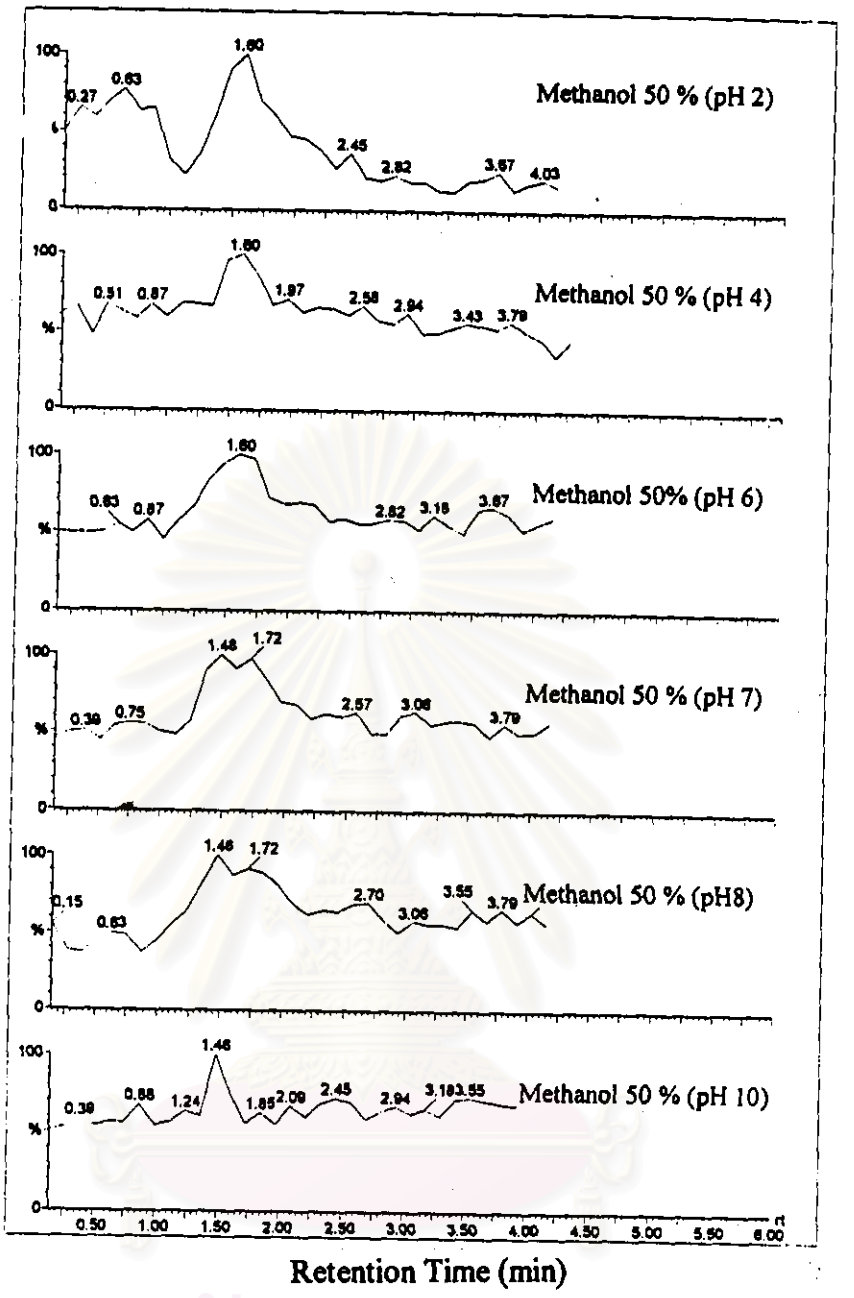


Figure 3.42 ESI SIR signal of 50 μL absolute methanol of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follow

Flow rate : 0.1 mL/min Lens 2 voltage : 210 V

Capillary tip voltage : 3.50 kV Lens 3 voltage : 10 V

Counter electrode voltage : 0.30 kV Source temperature : 80 ^{0}C

Cone voltage : 30 V

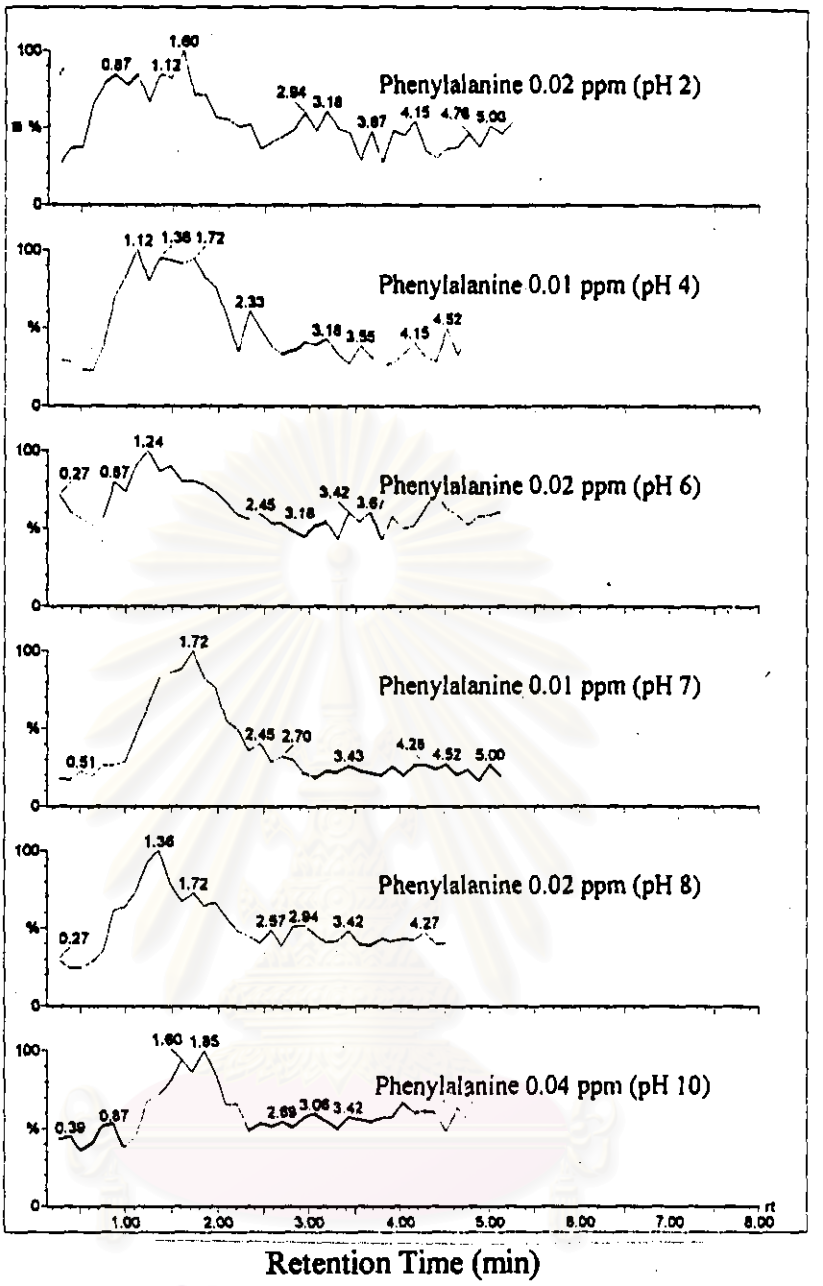


Figure 3.43 ESI SIR signal of 50 µL phenylalanine solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.1 mL/min Lens 2 voltage : 210 V

Capillary tip voltage : 3.50 kV Lens 3 voltage : 10 V

Counter electrode voltage : 0.30 kV Source temperature : 80 ^{0}C

Cone voltage : 30 V

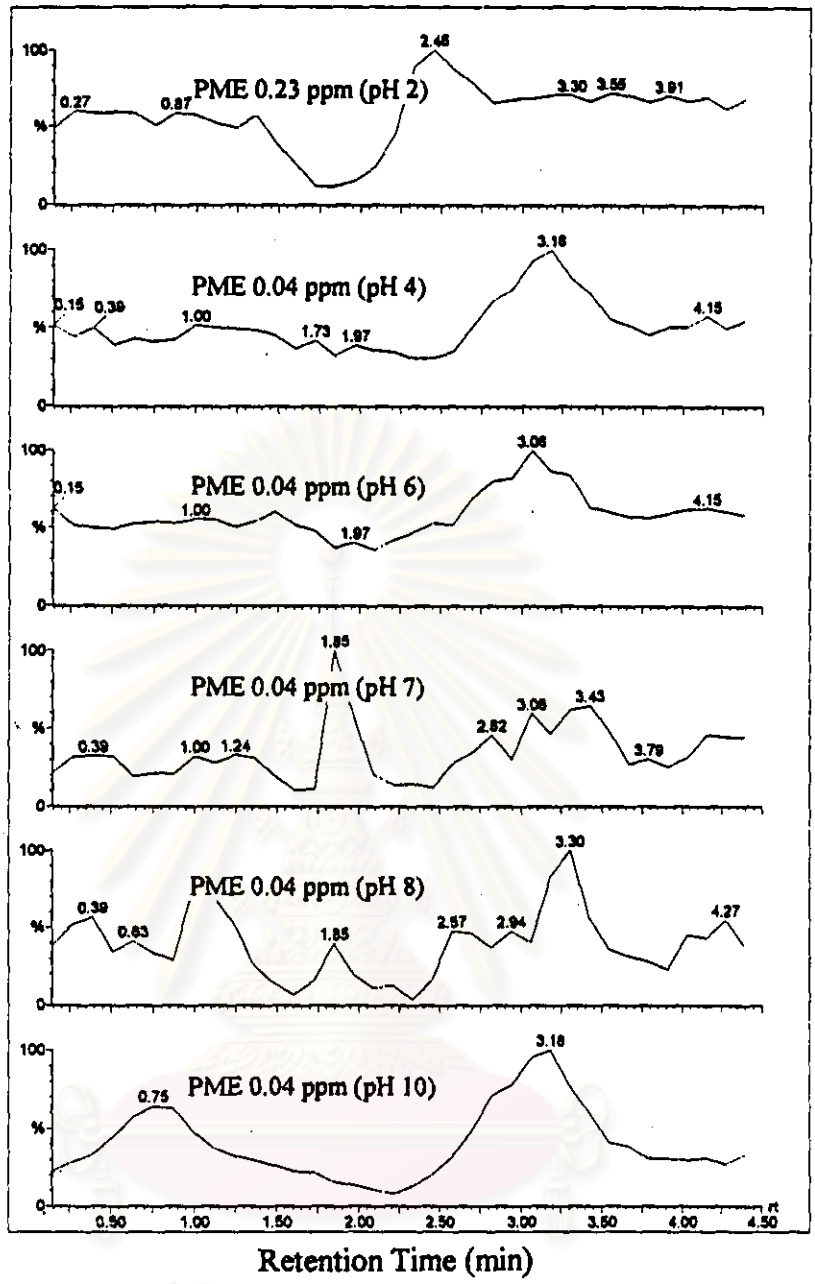


Figure 3.44 ESI SIR signal of 50 µL phenylalanine methyl ester solution of the lowest concentration possible to give the signal to noise ratio of ≥ 3 under each pH condition. All other parameters were as follows:

Flow rate : 0.1 mL/min Lens 2 voltage : 210 V

Capillary tip voltage : 3.50 kV Lens 3 voltage : 10 V

Counter electrode voltage : 0.30 kV Source temperature : 80 ^{0}C

Cone voltage : 30 V

Table 3.12 Detection limit of aspartame and its degradation products by APCI.

Compounds	Detection Limit at each pH in ppm unit (μg of the material required)					
	pH 2	pH 4	pH 6	pH 7	pH 8	pH 10
Aspartame	1.0 (0.05)	1.0 (0.05)	5.0 (0.25)	5.0 (0.25)	5.0 (0.25)	5.0 (0.25)
Aspartic acid	3.0 (0.15)	3.0 (0.15)	3.0 (0.15)	3.0 (0.15)	3.0 (0.15)	34 (1.7)
Aspartyl phenylalanine	6.0 (0.30)	6.0 (0.30)	6.0 (0.30)	6.0 (0.30)	10 (0.50)	50 (2.5)
Diketopiperazine	3.0 (0.15)	3.0 (0.15)	3.0 (0.15)	3.0 (0.15)	6.0 (0.30)	6.0 (0.30)
Methanol*	0.5	0.1	0.1	0.1	0.1	0.5
Phenylalanine	0.02 (0.001)	0.05 (0.003)	0.05 (0.003)	0.50 (0.025)	0.20 (0.01)	0.20 (0.01)
Phenylalanine methyl ester	0.05 (0.003)	0.05 (0.003)	0.05 (0.003)	0.05 (0.003)	0.05 (0.003)	0.05 (0.003)

*Numbers shown are concentration in % methanol in water (V/V).

Table 3.13 Detection limit of aspartame and its degradation products by ESI.

Compounds	Detection Limit at each pH in ppm unit (μg of the material required)					
	pH 2	pH 4	pH 6	pH 7	pH 8	pH 10
Aspartame	0.20 (0.010)	0.20 (0.010)	0.20 (0.010)	0.10 (0.005)	0.20 (0.010)	0.20 (0.010)
Aspartic acid	0.90 (0.045)	0.04 (0.002)	0.09 (0.004)	0.02 (0.001)	0.09 (0.004)	0.09 (0.004)
Aspartyl phenylalanine	0.30 (0.015)	0.50 (0.025)	0.10 (0.005)	0.50 (0.025)	0.50 (0.025)	0.20 (0.010)
Diketopiperazine	0.20 (0.010)	0.20 (0.010)	0.20 (0.010)	0.20 (0.010)	0.20 (0.010)	0.20 (0.010)
Methanol*	50	50	50	50	50	50
Phenylalanine	0.02 (0.001)	0.01 ($5x10^{-4}$)	0.02 (0.001)	0.01 ($5x10^{-4}$)	0.02 (0.001)	0.04 (0.002)
Phenylalanine methyl ester	0.23 (0.012)	0.04 (0.002)	0.04 (0.002)	0.04 (0.002)	0.04 (0.002)	0.04 (0.002)

*Numbers shown are concentration in % methanol in water (V/V).

From the above results, it is very obvious that ESI gave better sensitivity for most compounds tested (except methanol) than did the APCI technique. Detection of methanol was very poor in both techniques (~0.1 % (V/V) for APCI and 50 % (V/V) for ESI).

4. **Repeatibility Experiment**

In order to use the ESI technique for quantitative analysis of aspartame, the repeatibility of the technique was studied as to confirm the validity of this new method. The result of 15 injections of 10 ppm aspartame solution, 50 μL for each injection, is shown in Table 3.14. Repeatibility of the peak area is demonstrated here with the low relative standard deviation (RSD.), i.e., 0.0111 and low standard error (S.E.), i.e., 0.29.

Table 3.14 Repeatibility of peak of aspartame molecular peak obtained by ESI analysis (SIR at M/Z of 295). Standard 50 μL of aspartame solution was injected for each analysis.

No.	Peak Area
1	1810500
2	1819209
3	1856283
4	1838606
5	1836023
6	1849201
7	1832341
8	1845917
9	1853218
10	1846709
11	1831613
12	1830721
13	1815080
14	1800492
15	1785595
Mean*	1830100
S.D.*	20374.55
RSD.*	0.01113302
S.E.*	0.2874533

*see calculation in appendix

In order to compare the APCI with the ESI technique, repeatibility of APCI technique was tested. The result is shown in Table 3.15.

Table 3.15 Repeatibility of peak of aspartame molecular peak obtained by APCI analysis (SIR at M/Z of 295). Standard 50 μL of aspartame solution was injected for each analysis.

No.	Peak Area
1	1621
2	1473
3	1735
4	1429
5	1494
6	1510
7	1803
8	1585
9	1472
10	1003
11	1549
12	1264
13	1527
14	1811
15	1726
Mean	1533
S.D.	209.2
RSD.	0.1364
S.E.	3.522

5. **Comparison between APCI and ESI**

From Figure 3.9 and 3.24, it is very clear that under optimal condition (condition that gave the highest sensitivity for aspartame molecular peak), ESI technique gave a much cleaner spectrum than APCI did. The ESI spectrum of aspartame shows no fragmentation of molecular ion, it is, therefore suitable for the

next study in which various aspartame's degradation products must be monitored simultaneously. The facts that there is no fragmentation of aspartame under ESI enables us to monitor aspartame's degradation products without the need to worry if peaks detected belongs to the degradation products present in the sample or arised from fragmentation of aspartame parent ions during ionization process in the ESI source.

Moreover, ESI technique gave a better sensitivity than APCI for aspartame detection (detection limit of 0.2 ppm for ESI and 5.0 ppm for APCI).

Results in Part I can be concluded as shown in Table 3.16. ESI-MS was the superior API technique and this technique of ionization was used in Part II study.

Since the result from APCI-MS shows fragmentation of all the compounds tested, this method can, therefore, be used effectively as a detector in liquid chromatography for confirmation of chemical structures.

Table 3.16 Comparison between ESI and APCI for the analysis of aspartame and its degradation products.

	ESI	APCI
Fragmentation pattern	$(M+1)^+$ ion is the base peak for all compounds tested with negliTable fragmentation.	Prominent fragmentation present for most compounds tested. Base peak was not always the molecular peak.
Detection Limit	Lower for all compounds tested except methanol.	Higher for all compounds tested except methanol.
Repeatibility	Better	Worse

PART II: ASPARTAME STABILITY AND ITS DEGRADATION CHEMISTRY.

From Part I, it is clear that ESI-MS can be used to detect aspartame, aspartic acid, aspartyl phenylalanine, diketopiperazine, phenylalanine and phenylalanine methyl ester. Although methanol can not be directly monitored by this technique, detection of other degradation products would be enough to draw out the degradation pathway of aspartame. To moniter the present of aspartame, aspartic acid, aspartyl phenylalanine, diketopiperazine, phenylalanine and phenylalanine methyl ester in the tested solution simultaneously, ESI-MS must be set in SIR mode to record molecular ions at M/Z of 295, 134, 281, 263, 166 and 180, respectively.

Figure 3.24 - 3.30 in Part I demonstrated that all the compounds ran under the optimal condition did not fragment during the electrospray ionization process, therefore, detection of molecular ions of aspartame degradation products would directly indicate the presences of those compounds in the tested solution.

In order to test how stable aspartame is at various pH and to monitor how aspartame degrades under various pH along a period of times, aspartame and all its degradation products in solution of various pH must be monitored quantitatively at various time. There are two ways to achive this quantitative analyses, one is to perform the analyses of the kept standard solution together with the calibration experiments for each of every compound being monitored everytime when the solution is being tested, the other way is to use the relative sensitivity of each compound as a correction factor to calculate the relative abundance of each compound in the tested

solution. In this study, the second strategy was used since 1) there were 6 compounds being monitored and to do the caliblation curves for each of them everytime would be too much work and 2) testing of the second strategy, indicated a good reliability.

To get the correction factor for each compound, standard solution containing six compounds was prepared to yield exact same concentration of each compound. Fifty microlitres of this solution were injected into the ESI-MS using optimal condition obtained in Part I, SIR at M/Z of corresponding molecular ions were done. This experiment was repeated for 15 times. Integration of peak area gave integration number which was converted to relative signal compared to aspartame signal, i.e., aspartame peak area was set as 1 (table 3.17). The number gives the idea of how sensitive that compound is under ESI analysis when compared to aspartame. The reciprocal of each number was the correction factor (C.F.) used to multiply the corresponding signal obtained from the tested solution.

Table 3.17 Correction factor of 6 standards.

No.	Relative Sensitivity of the Compound Compared to Aspartame = Peak Area of Compound/Peak Area of Aspartame					
	Asp	Phe	PME	DKP	Asp-Phe	Aspartame
1	0.25	1.11	4.14	0.04	0.28	1
2	0.29	1.21	4.50	0.04	0.31	1
3	0.22	1.06	4.01	0.04	0.31	1
4	0.25	1.07	4.03	0.04	0.29	1
5	0.26	1.13	4.30	0.04	0.30	1
6	0.26	1.02	4.14	0.04	0.30	1
7	0.27	1.08	4.13	0.04	0.31	1
8	0.29	1.11	4.23	0.04	0.31	1
9	0.26	1.04	4.14	0.04	0.30	1
10	0.27	1.07	4.07	0.05	0.30	1
11	0.29	1.25	5.04*	0.06	0.31	1
12	0.24	0.88	3.84	0.04	0.31	1
13	0.23	0.99	3.97	0.04	0.31	1
14	0.23	0.93	3.96	0.04	0.30	1
15	0.25	0.99	3.82	0.04	0.30	1
MEAN(X)	0.25733	1.06267	4.09143	0.042	0.30267	1
C.F. = 1/X	3.88601	0.94103	0.24441	23.8095	3.30396	1

* = rejected by Q-test

Solution containing 10 ppm of compounds were tested to obtain correction factor by the same procedure, the results show no different in relative sensitivity.

After correction factor of each compound was obtained, the study of aspartame stability and how it was degraded into (degradation pathway) at various pH was performed.

Fifty microlitres of 10 ppm standard aspartame solution prepared at pH of 2, 4, 6, 7, 8 and 10 was withdrawn and analyzed by ESI-MS every 5 days. The results are shown as graph representing relative abundance of each of the six componunds versus the age of solution. From graphs in Figure 3.45 - 3.50, degradation pathway of aspartame at various pH can be drawn as shown in Figure 3.51 - 3.53.

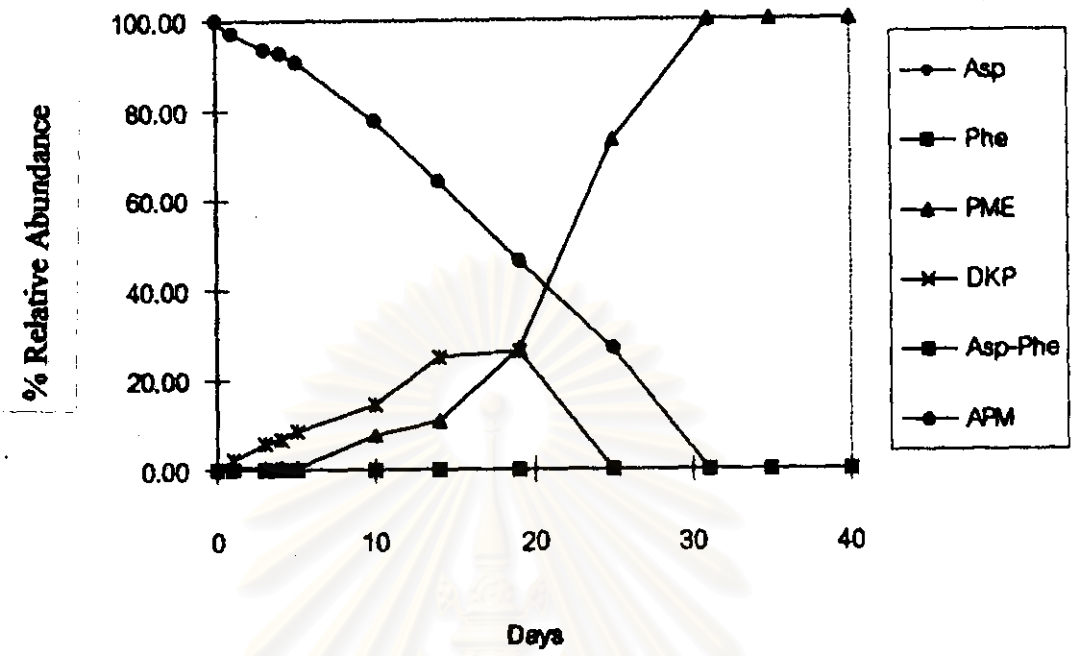


Figure 3.45 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the standard aspartame solution (pH 2) was kept for 40 days. Each point on the graph was the average of at least four experimental values.

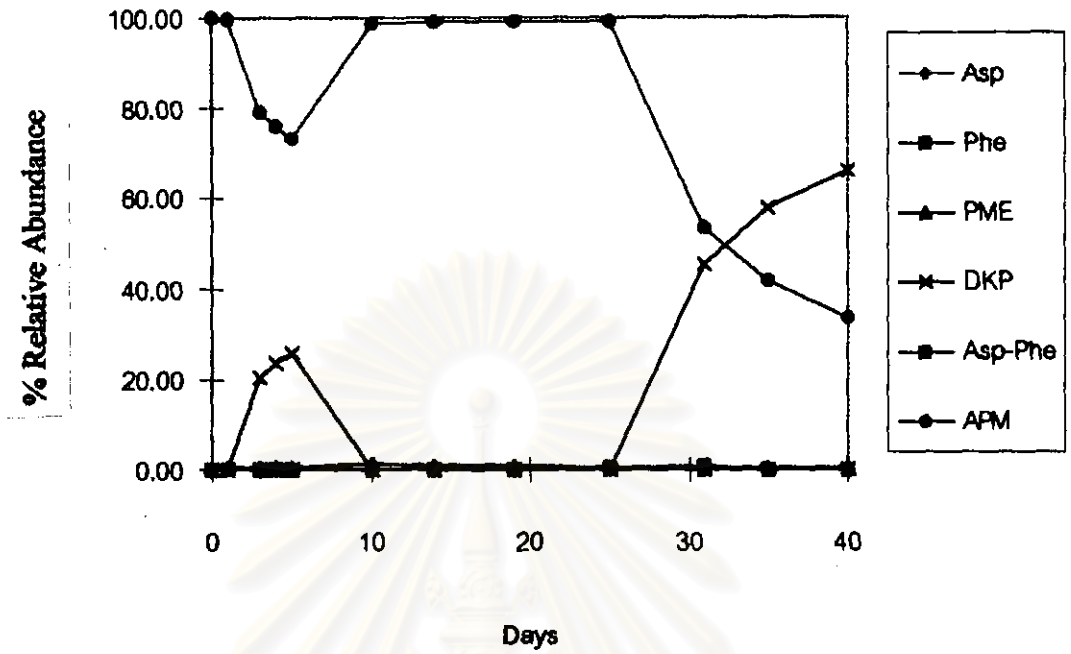


Figure 3.46 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the standard aspartame solution (pH 4) was kept for 40 days. Each point on the graph was the average of at least four experimental values.

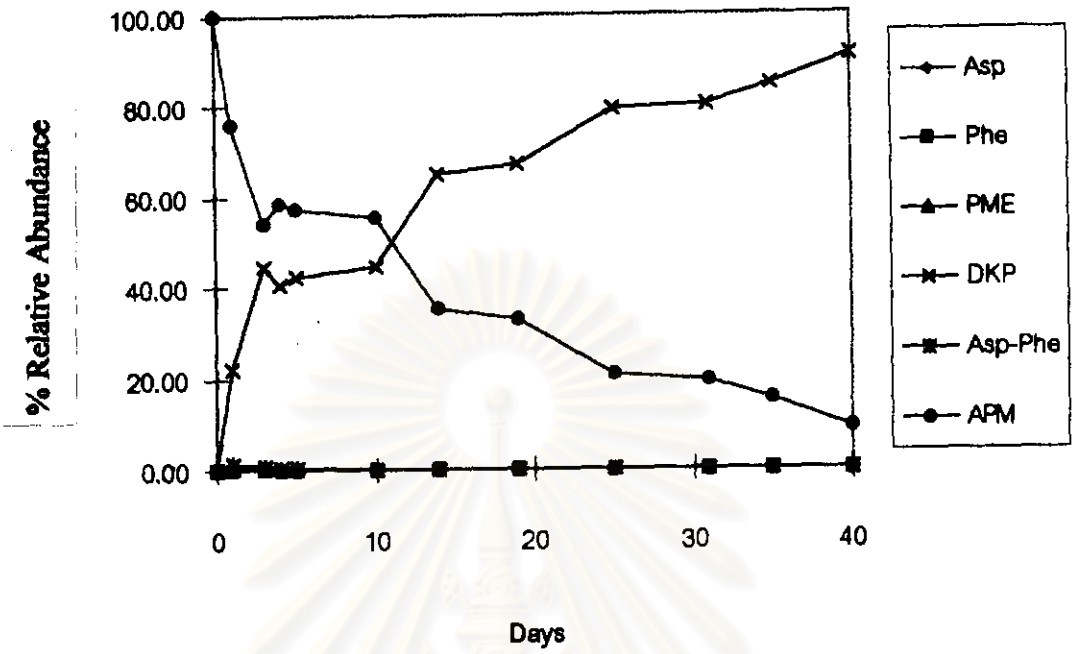


Figure 3.47 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the standard aspartame solution (pH 6) was kept for 40 days. Each point on the graph was the average of at least four experimental values.

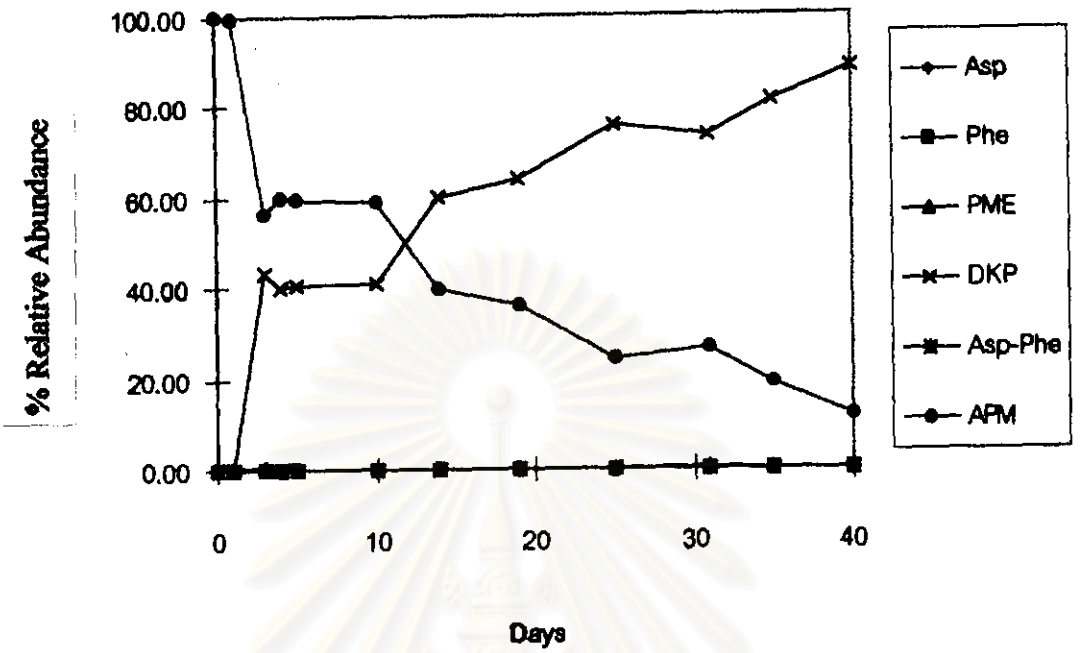


Figure 3.48 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the standard aspartame solution (pH 7) was kept for 40 days. Each point on the graph was the average of at least four experimental values.

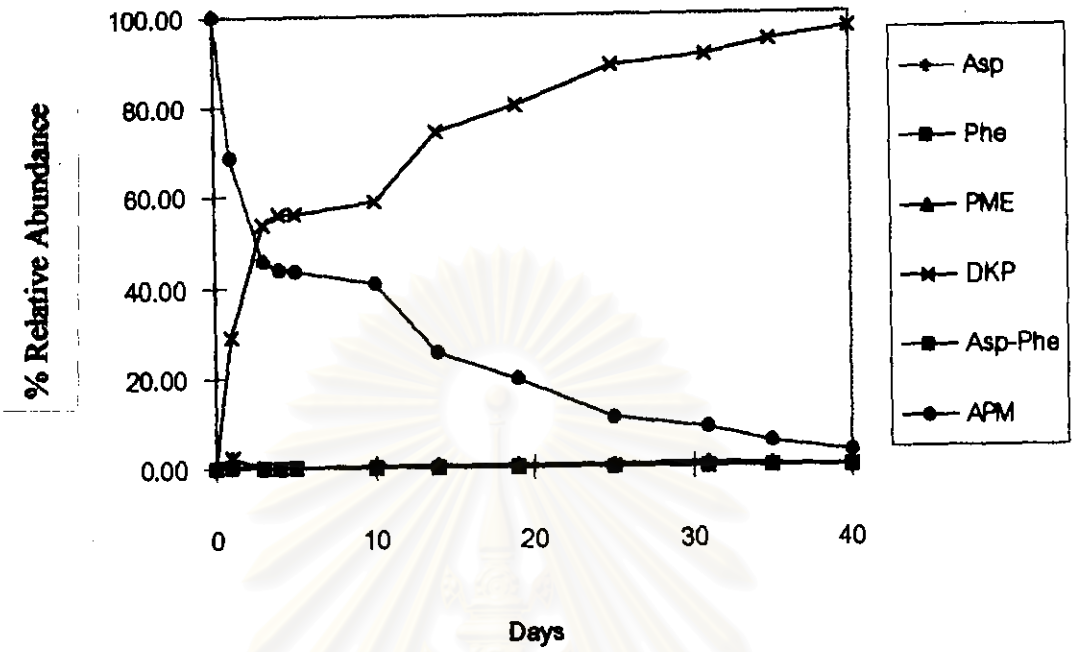


Figure 3.49 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the standard aspartame solution (pH 8) was kept for 40 days. Each point on the graph was the average of at least four experimental values.

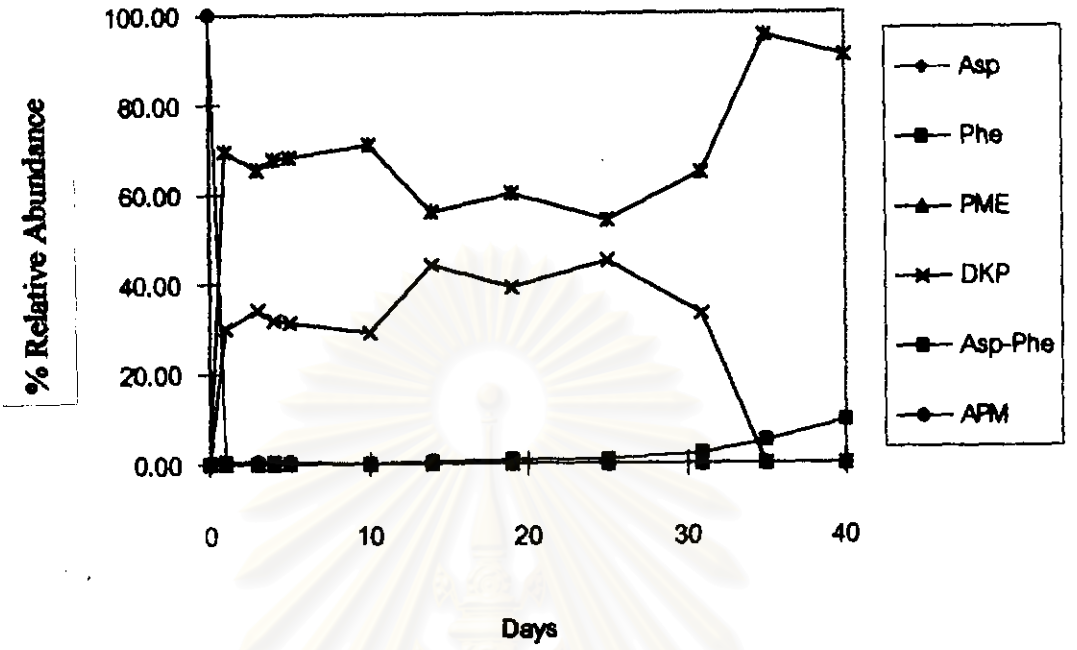


Figure 3.50 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the standard aspartame solution (pH 10) was kept for 40 days. Each point on the graph was the average of at least four experimental values.

At pH 2, aspartame was slowly degraded into aspartyl phenylalanine (release of methanol) and phenylalanine methyl ester (release of aspartic acid) (Figure 3.45). Methanol and aspartic acid were both, however, undetected from the experiment. This was probably because, the concentration of the two species were too low (lower than detection limit). When kept for more than 20-25 days aspartyl phenylalanine

disappeared from the solution, it was probably degraded into undetectable compounds. Phenylalanine methyl ester was the major products found when kept for 40 days.

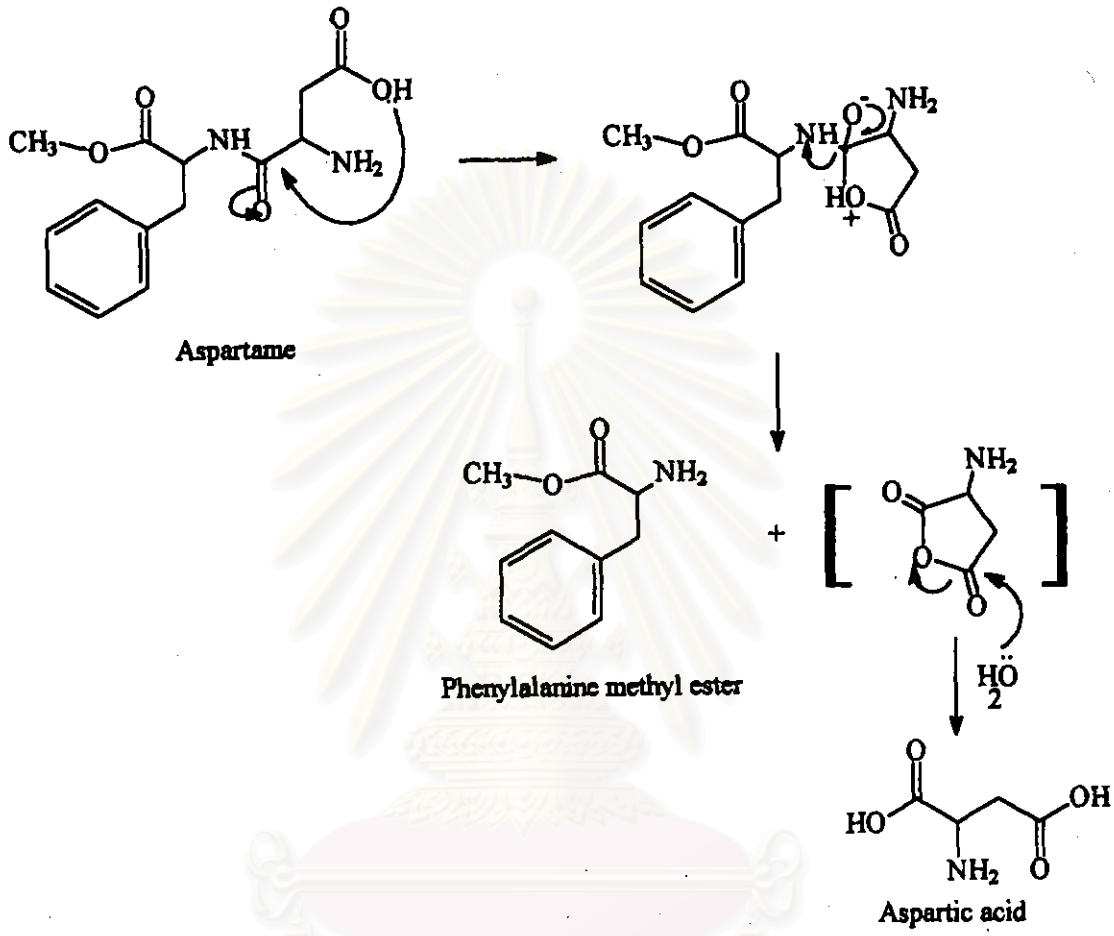


Figure 3.51 Degradation pathway of aspartame to phenylalanine methyl ester.

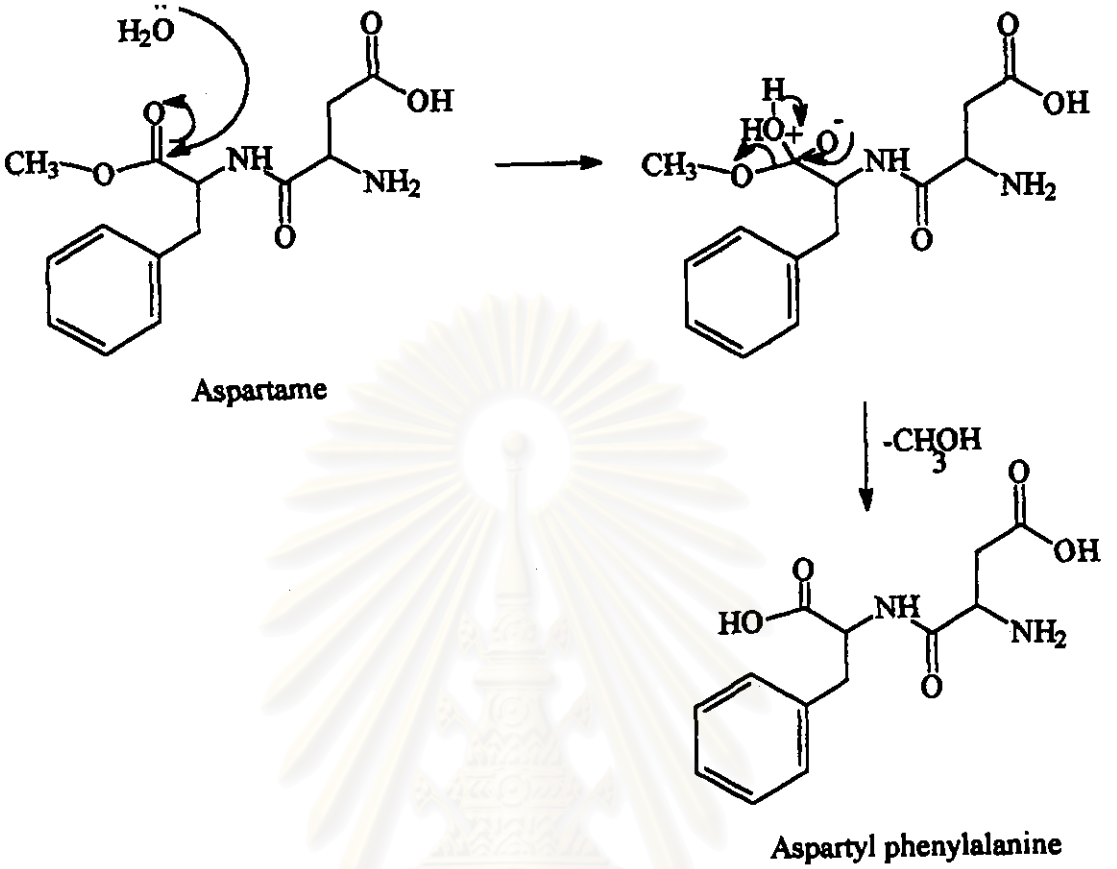


Figure 3.52 Degradation pathway of aspartame to aspartyl phenylalanine.

For the pH 4 solution, the only degradation product detected was diketopiperazine. The proposed degradation pathway of aspartame to diketopiperazine was shown in Figure 3.53.

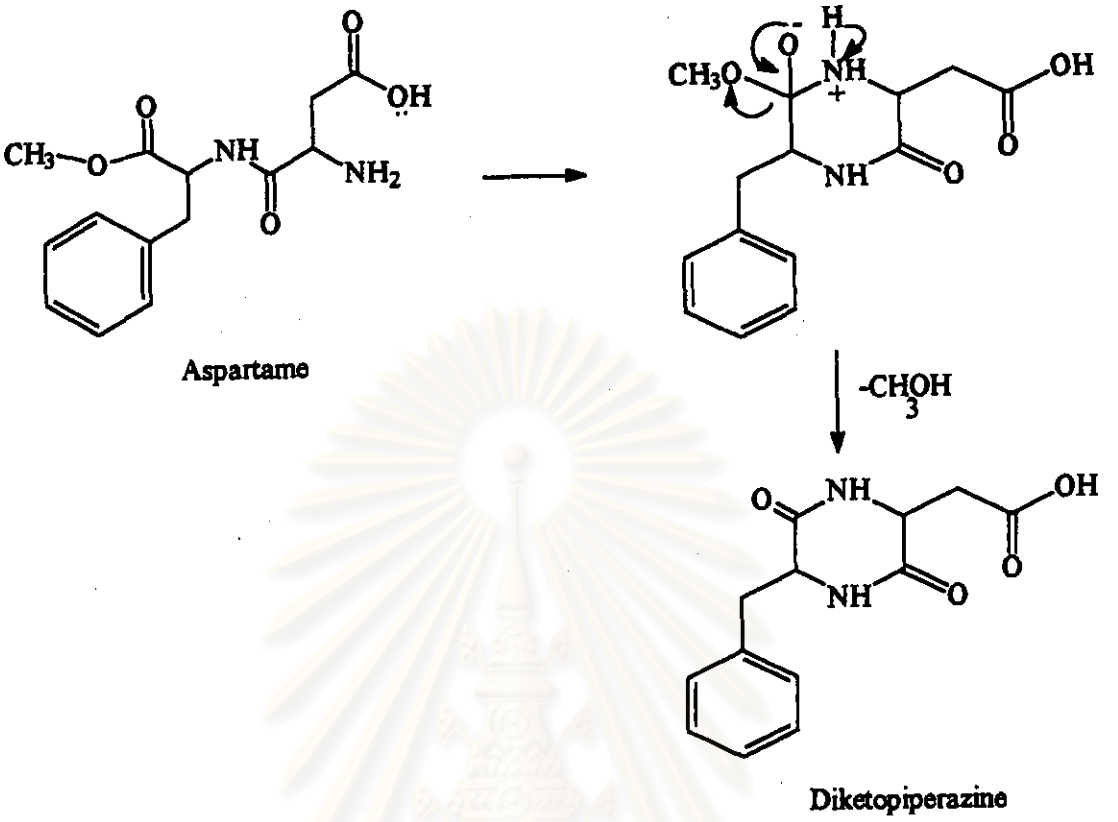


Figure 3.53 Degradation Pathway of aspartame to diketopiperazine.

At pH 6, 7 and 8, the main degradation product detected was diketopiperazine. Comparing the rate of degradation for these pH, it was found that the rate was highest for the pH 8 and lowest for the pH 6. Degradation pathway from aspartame to DKP is shown in Figure 3.53.

The two degradation products detected in the pH 10 solution were aspartyl phenylalanine and diketopiperazine. Aspartame degraded very quickly under this very basic condition. There was no aspartame after 1 day. Phenylalanine was also detected after 25 days.

The above results indicate different degradation pathways for aspartame kept at different pH.

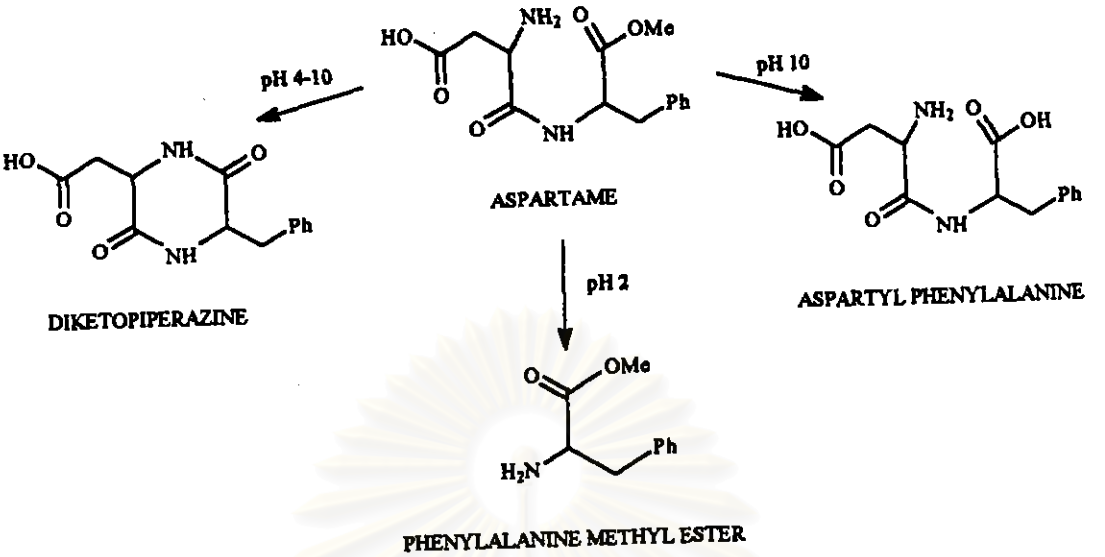


Figure 3.54 Summary of aspartame degradation pathways.

To make this study to be more completed, one should start with a higher concentration of standard aspartame, i.e., 500 ppm instead of 10 ppm so as to all other degradation products will be detectable.

After realizing of this fact, 500 ppm standard aspartame solution were prepared at various pH and kept at room temperature. Results of the analysis of these solutions are shown in Figure 3.55-3.60.

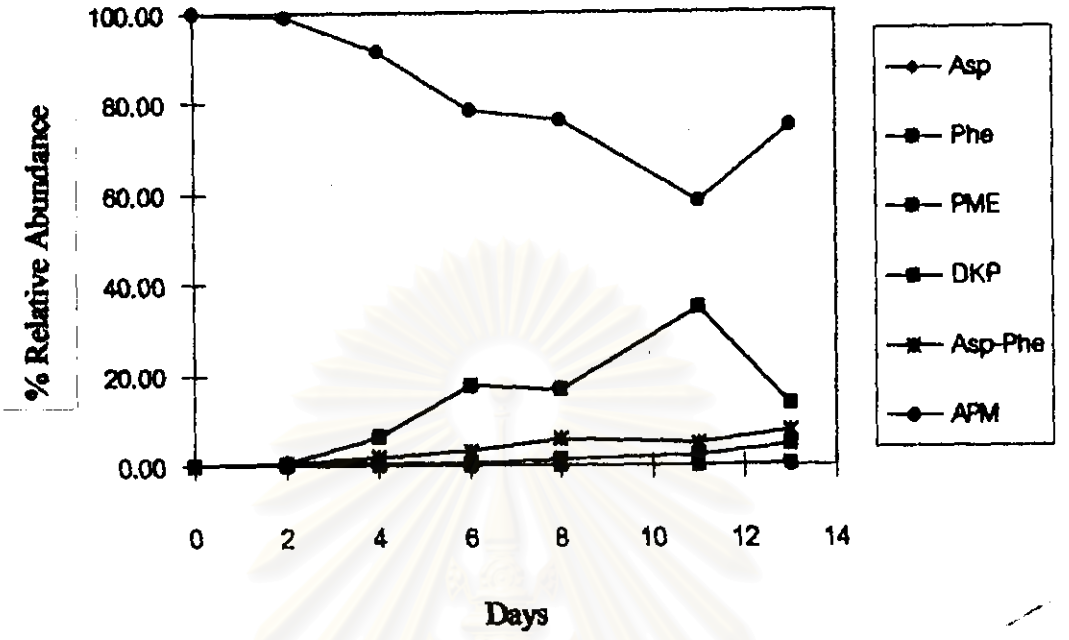


Figure 3.55 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the 500 ppm standard aspartame solution (pH 2) was kept for 13 days. Each point on the graph was the average of at least four experimental values.

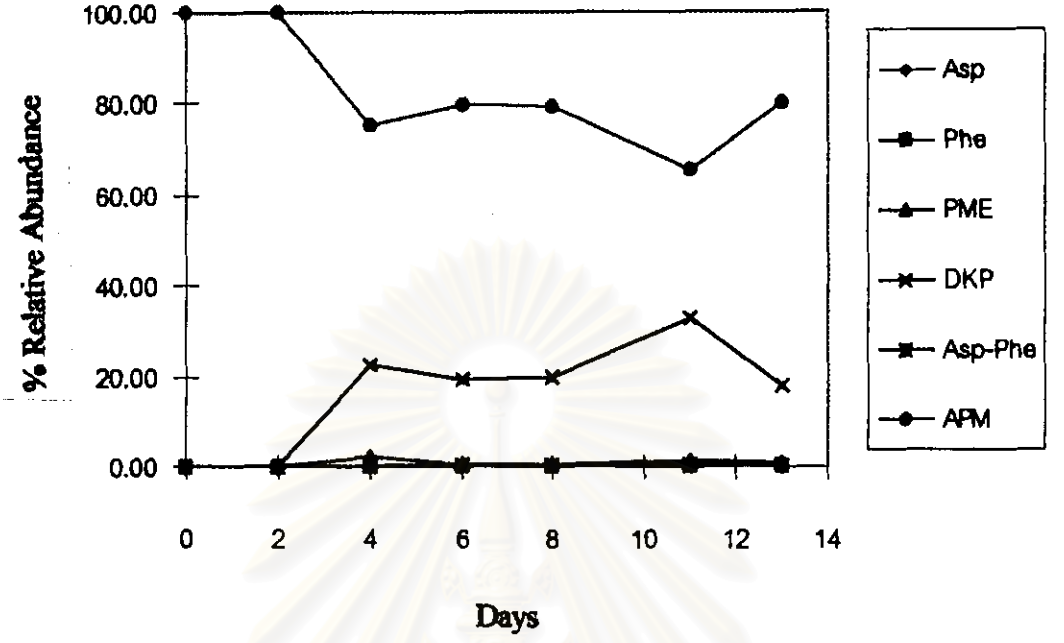


Figure 3.56 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the 500 ppm standard aspartame solution (pH 4) was kept for 13 days. Each point on the graph was the average of at least four experimental values.

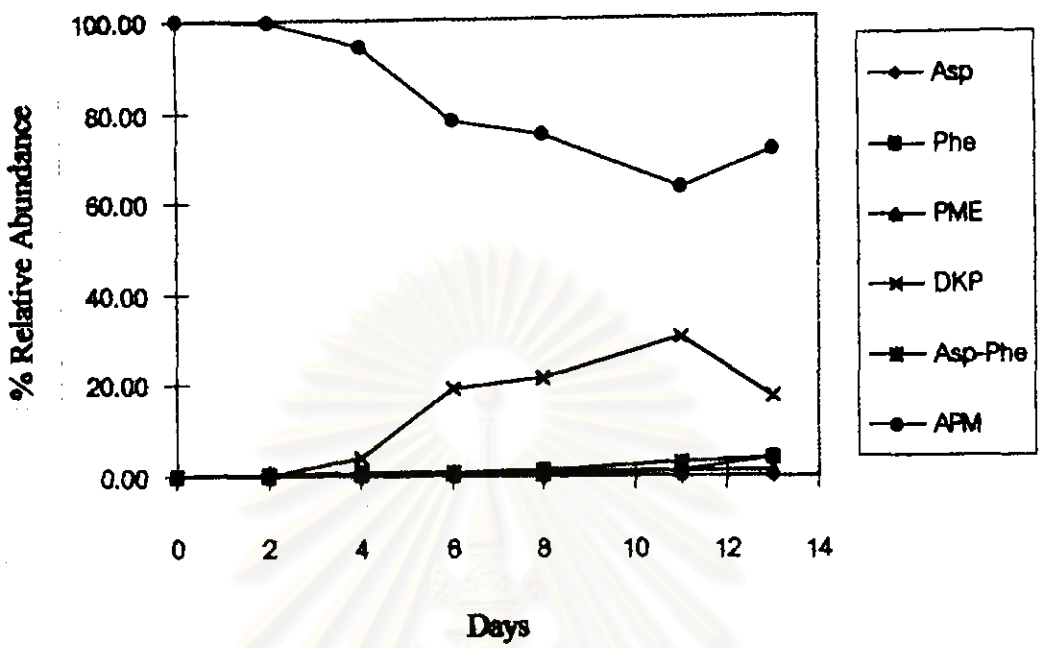


Figure 3.57 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the 500 ppm standard aspartame solution (pH 6) was kept for 13 days. Each point on the graph was the average of at least four experimental values.

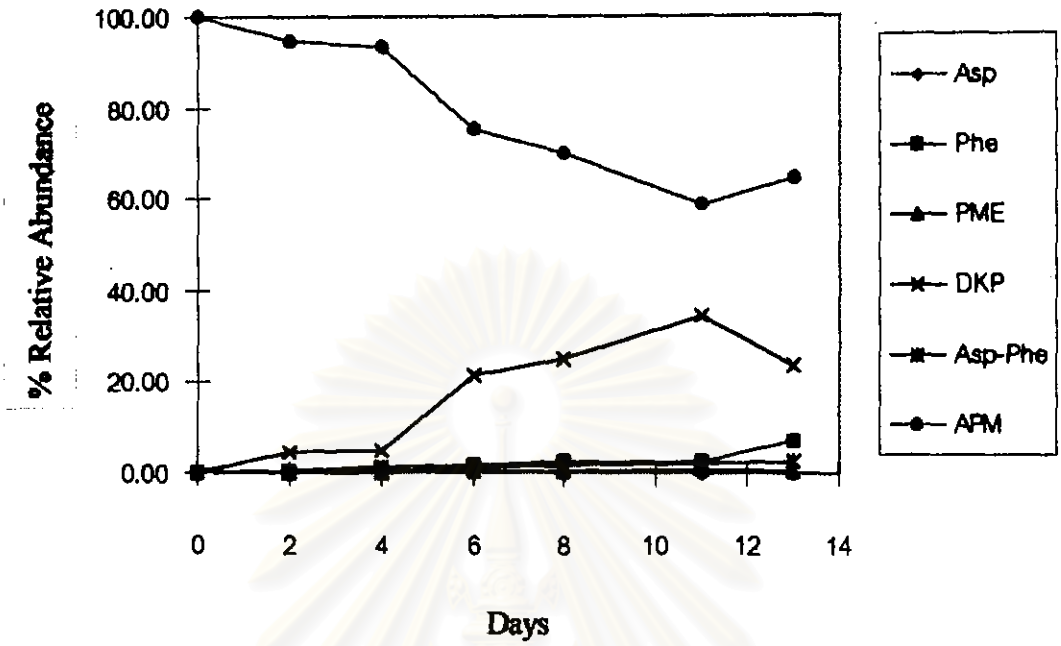


Figure 3.58 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the 500 ppm standard aspartame solution (pH 7) was kept for 13 days. Each point on the graph was the average of at least four experimental values.

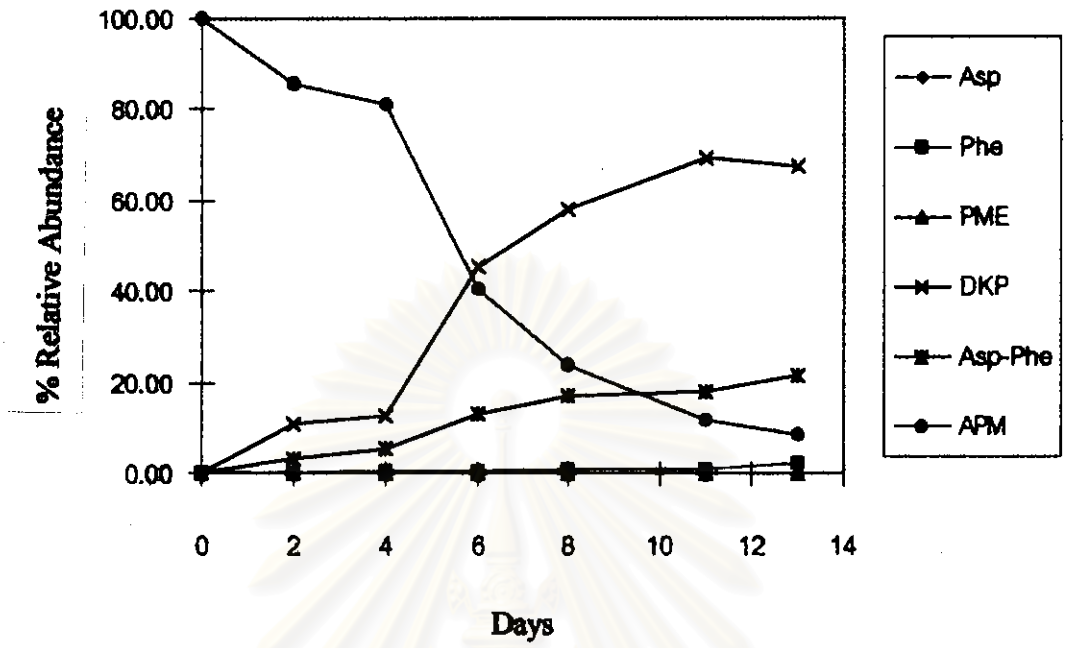


Figure 3.59 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the 500 ppm standard aspartame solution (pH 8) was kept for 13 days. Each point on the graph was the average of at least four experimental values.

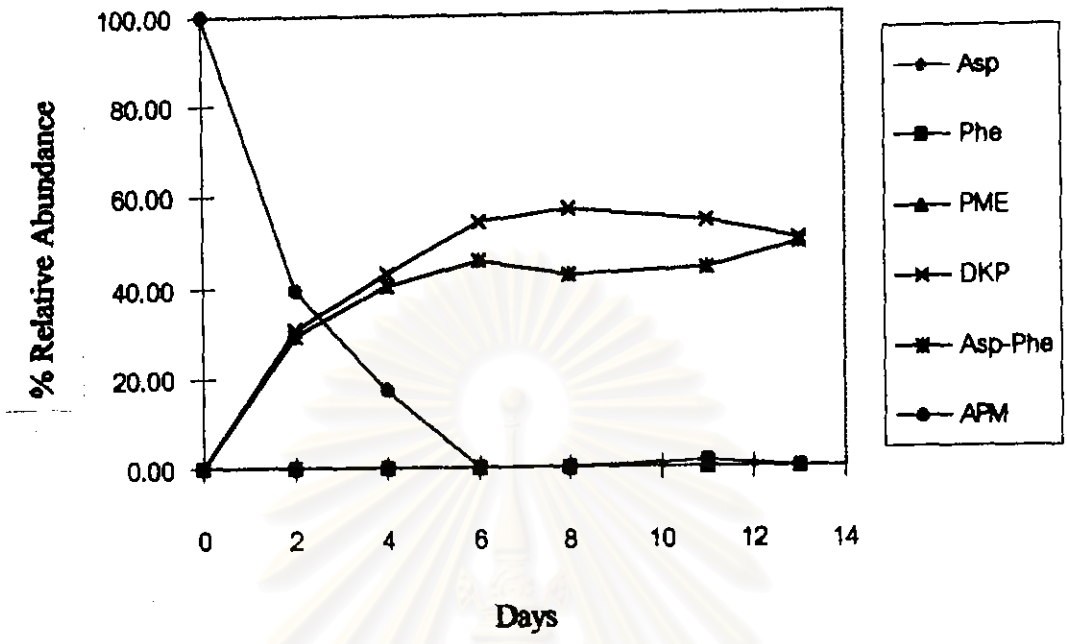


Figure 3.60 Progress curve representing the changes in relative abundance of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) as the 500 ppm standard aspartame solution (pH 10) was kept for 13 days. Each point on the graph was the average of at least four experimental values.

Stability of aspartame solution in different temperature

The 10 ppm aspartame solutions were kept at 4, 30 (room temperature) and 80 ^{o}C for 10 days. Each solution was withdrawn and analyzed for aspartame, aspartic acid, aspartyl phenylalanine, diketopiperazine, methanol, phenylalanine and phenylalanine methyl ester by ESI-MS. The results are shown in Figure 3.61a, b and c.

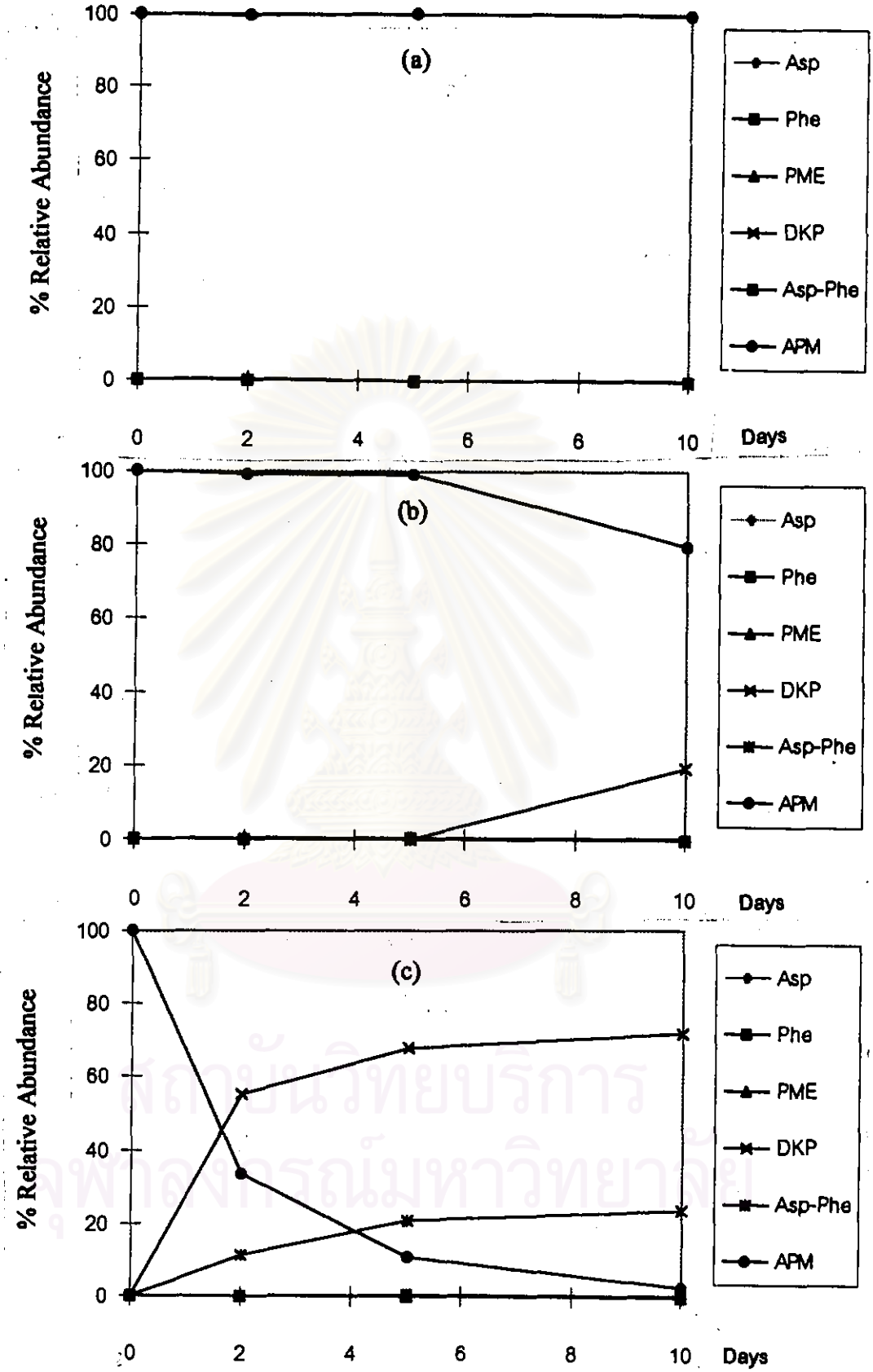


Figure 3.61 Relative abundance of aspartame and its degradation products in standard aspartame solution kept at a) 4 ^{0}C b) 30 ^{0}C and c) 80 ^{0}C.

From Figure 3.61a, it can be seen that aspartame was rarely degraded if kept at 4 ^{0}C. This was concluded from the fact that there was no degradation products detected in the solution which was kept for 10 days.

If kept at room temperature for 10 days, DKP was the major degradation products detected (Figure 3.61b). This indicates some degradation of aspartame.

When the solution was kept at 80 ^{0}C for 10 days, the major degradation products were diketopiperazine and aspartyl phenylalanine (Figure 3.61c). Phenylalanine and aspartic acid were also detected.

Determination of Aspartame and Its Degradation Products in Samples

The calibration curves of all six standards were used for calculating the concentration of aspartame and its degradation products (Figure 3.62 - 3.67). The fitting of the curves was done by least square method.

Soft drinks used in this study included Diet 7-Up, Diet Coke, Diet Pepsi and Pepsi Max. These soft drinks were degassed before injection. Soft drinks samples were tested to monitor aspartame degradation. Concentration of aspartame (APM), aspartic acid (Asp), aspartyl phenylalanine (Asp-Phe), diketopiperazine (DKP), phenylalanine (Phe) and phenylalanine methyl ester (PME) in fresh soft drinks and stocked soft drinks (8-10 months) were compared. The results were shown in Table 3.18.

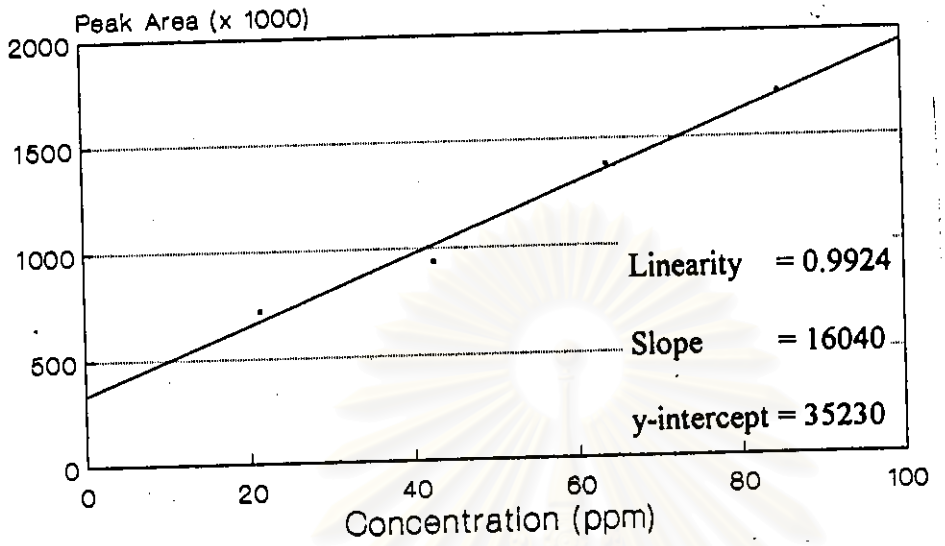


Figure 3.62 Calibration curve of aspartame.

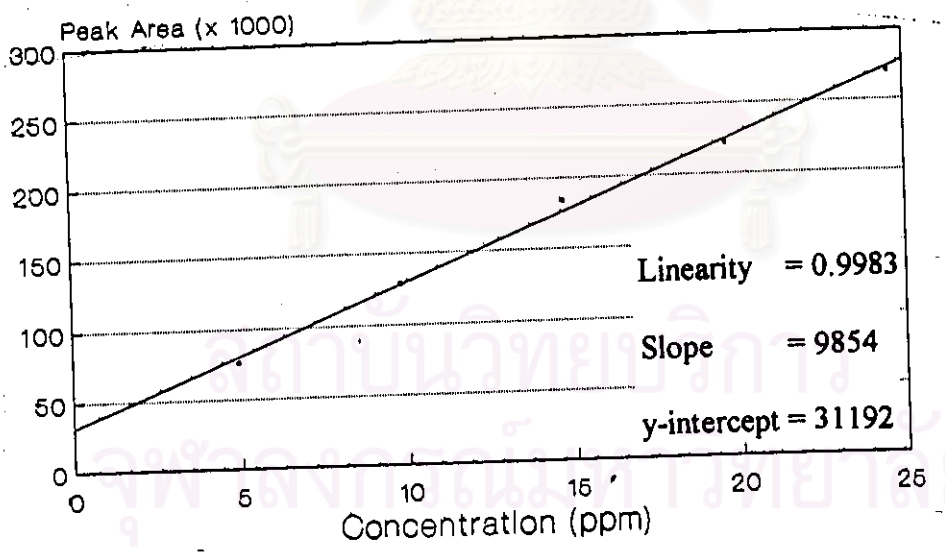


Figure 3.63 Calibration curve of aspartic acid.

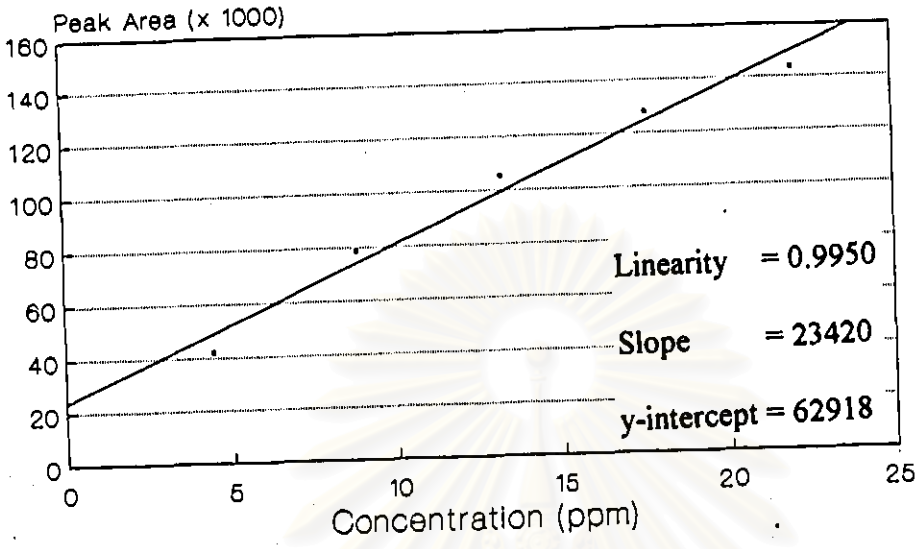


Figure 3.64 Calibration curve of aspartyl phenylalanine.

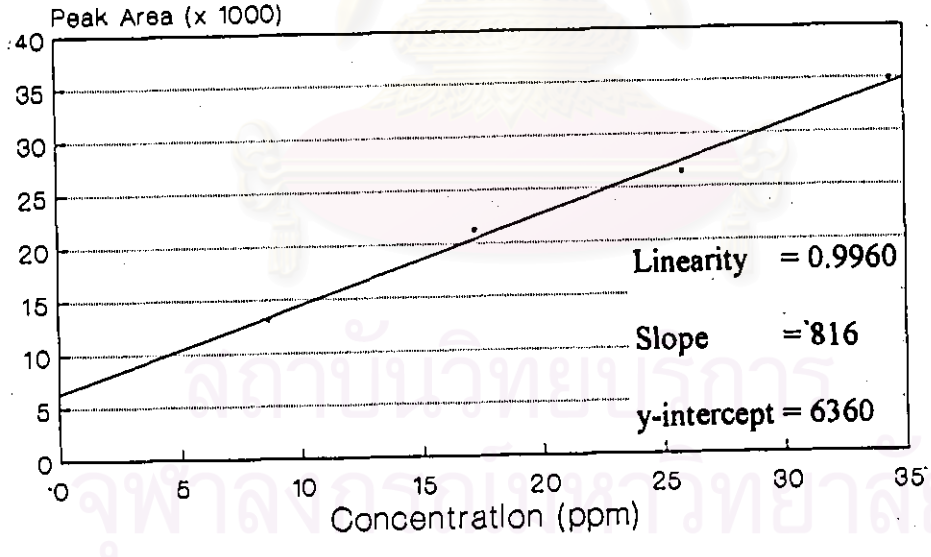


Figure 3.65 Calibration curve of diketopiperazine.

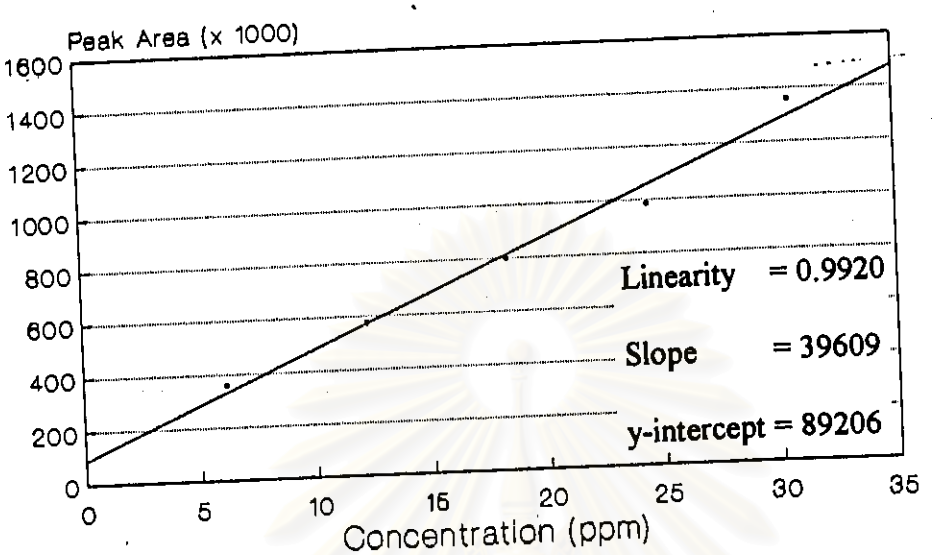


Figure 3.66 Calibration curve of phenylalanine.

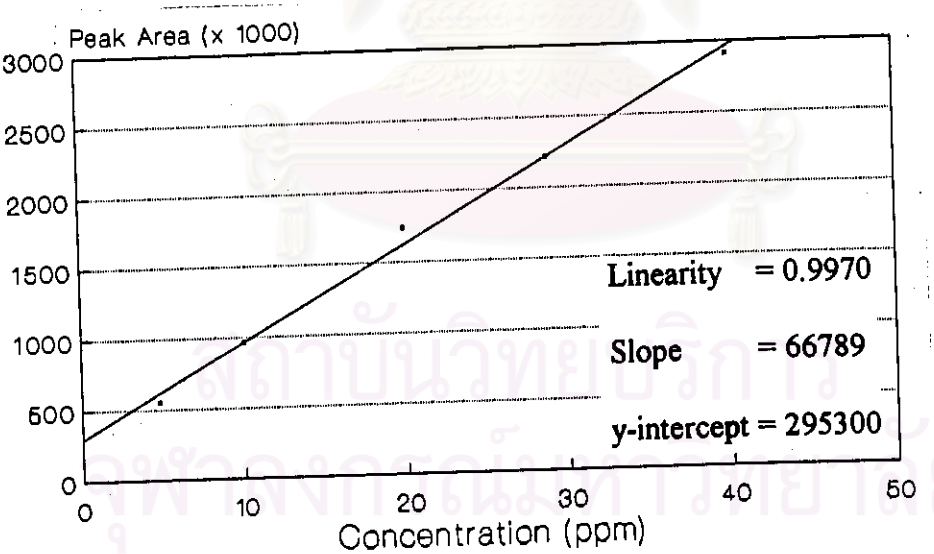


Figure 3.67 Calibration curve of phenylalanine methyl ester.

Table 3.18 Concentration of aspartame and its degradation products in soft drinks kept for certain period at room temperature.

Soft drinks	Concentration (ppm)					
	Asp	Phe	PME	DKP	Asp-Phe	APM
Diet 7-Up	0.00	0.00	0.00	0.00	0.00	505.83 ±0.30
Diet 7-Up (10 months)	0.00	4.72 ± 0.06	4.97 ± 0.52	26.30 ± 0.42	1.35 ± 2.28	84.64 ± 0.03
Diet Coca-cola	0.00	0.00	0.00	1.09 ± 5.29	0.00	281.87 ± 2.35
Diet Coca-cola (8 months)	0.00	1.10 ± 1.01	3.05 ± 1.20	20.38 ± 0.35	0.19 ± 4.63	48.02 ± 0.66
Diet Pepsi	0.00	0.00	0.00	3.32 ± 0.83	0.00	462.30 ± 2.54
Diet Pepsi (10 months)	0.00	7.75 ± 10.72	7.63 ± 0.85	18.05 ± 1.31	1.70 ± 6.40	51.61 ± 1.41
Pepsi Max	0.00	0.00	0.00	0.00	0.00	479.97 ± 1.62
Pepsi Max (10 months)	2.31 ± 0.93	12.48 ± 2.12	12.31 ± 0.85	26.01 ± 1.31	3.21 ± 6.40	58.02 ± 1.41

From the results in Table 3.18, it was obvious that aspartame in fresh soft drinks was rarely degraded. But if soft drinks were kept at room temperature for long times

aspartame will degrade into diketopiperazine, phenylalanine, phenylalanine methyl ester, aspartyl phenylalanine and aspartic acid. Diketopiperazine was the main degradation products. Although methanol was not qualitated directly by the method (its concentration was beyond the detection limit), the result which showing the presences of diketopiperazine and/or aspartyl phenylalanine and/or phenylalanine indicates the release of methanol upon the degradation process.

Dry products used in this study were Equal, Fitne and Slimma. These three sweeteners were separately dissloved in distilled water at room temperature and distilled water at 100 ^{0}C. The solution was analyzed by ESI-MS after 10 min of preparation. The condition of 100 ^{0}C water was used in order to imitate the condition normally used to prepare hot coffee and tea.

Table 3.19 Concentration of aspartame and its degradation products in dry products.

Samples	Concentration (ppm)					
	Asp	Phe	PME	DKP	Asp-Phe	APM
Equal in water	0.00	0.00	0.00	0.00	0.00	186.18 ± 6.06
Equal in hot water	0.00	0.00	0.00	0.00	0.00	175.22 ± 1.60
Fitne in water	0.00	0.00	0.00	0.00	0.00	161.96 ± 3.66
Fitne in hot water	0.00	0.00	0.00	0.00	0.00	146.38 ± 3.12
Silmma in water	0.00	0.00	0.00	0.00	0.00	204.47 ± 2.17
Slimma in hot water	0.00	0.00	0.00	0.00	0.00	173.42 ± 4.20

From Table 3.19, it can be seen clearly that there was no degradation products detected in these products. It should be noted here that each sample was prepared by adding one envelope of the products in 200 mL of hot-or room temperature-water. The concentration differences between hot and room temperature solution (as shown in the Table) is likely to be due to the differences at the starting amount of sample.

The fact that there was no significant amount of degradation products detected confirms that there is no significant degradation of aspartame in hot water .