CHAPTER IV

RESULTS AND DISCUSSION

4.1 Properties of Glyphosate and AMPA and Preliminary Experiment

Before describing the experimental results, the analytes properties should be considered in the extraction mechanism theme. The pK_a values for glyphosate are 0.8, 2.3, 6.0 and 11.0 and for AMPA are 0.9, 5.6 and 10.2, respectively. Glyphosate solubility in water is 11,600 ppm (at 25 °C) with octanol-water coefficient (log K_{ow}) of -3.5. The values indicate that its intrinsic properties are very hydrophilic and easily dissolves in aqueous solution. Its very low octanol-water coefficient indicates that organic solvent extraction such as LLE and two-phase membrane technique will not work at extracting the analyte from aqueous media. However, three-phase membrane technique that used organic solvent can not be used directly; a carrier-mediated technique is required to facilitate the mass transport.

Glyphosate and its metabolite have acid and base characteristics. Their pK_a values suggest that glyphosate and AMPA are polyprotic. The dissociation equilibria are shown in Figure 4.1 and Figure 4.3.

The dissociation fraction (∝) of each species of polyprotic acid H_nA at a given pH can be calculated by fractional composition equation:

$$\alpha_{H^{n-j}A} = \frac{K_1 K_2 \ldots K_j \left[H^+\right]^{n-j}}{D} \qquad \text{(Eq. 10)}$$

where K = equilibrium constant

$$D = [H^+]^n + K_1[H^+]^{n-1} + K_1K_2[H^+]^{n-2} + \ldots + K_1K_2K_3\ldots K_n$$

From equation (10), we can predict the fraction of glyphosate and AMPA species at a given pH as present in the diagrams (Figure 4.2 and Figure 4.4) below.

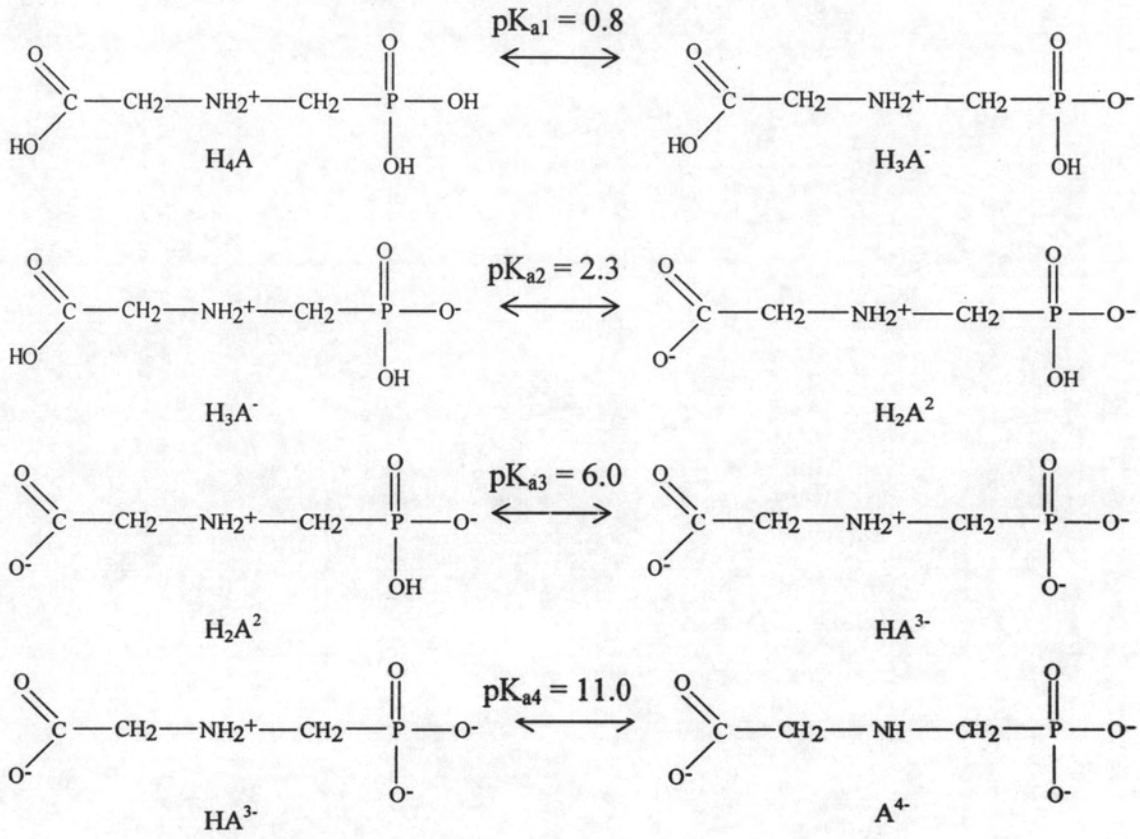

Figure 4.1 Glyphosate equilibria.

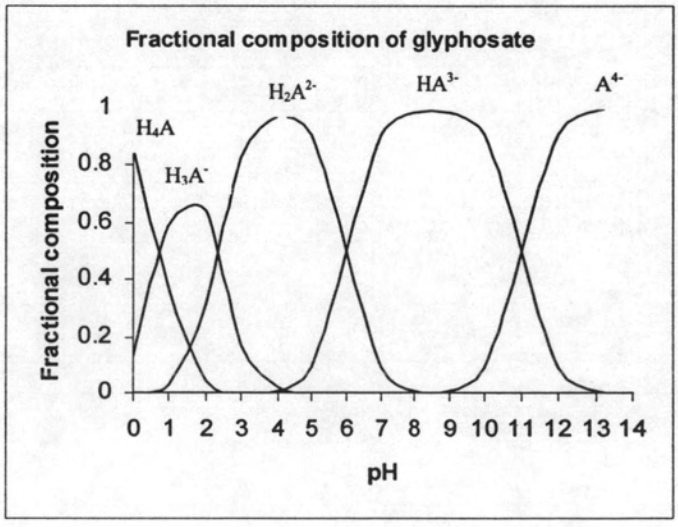

Figure 4.2 Fractional composition diagram of glyphosate.

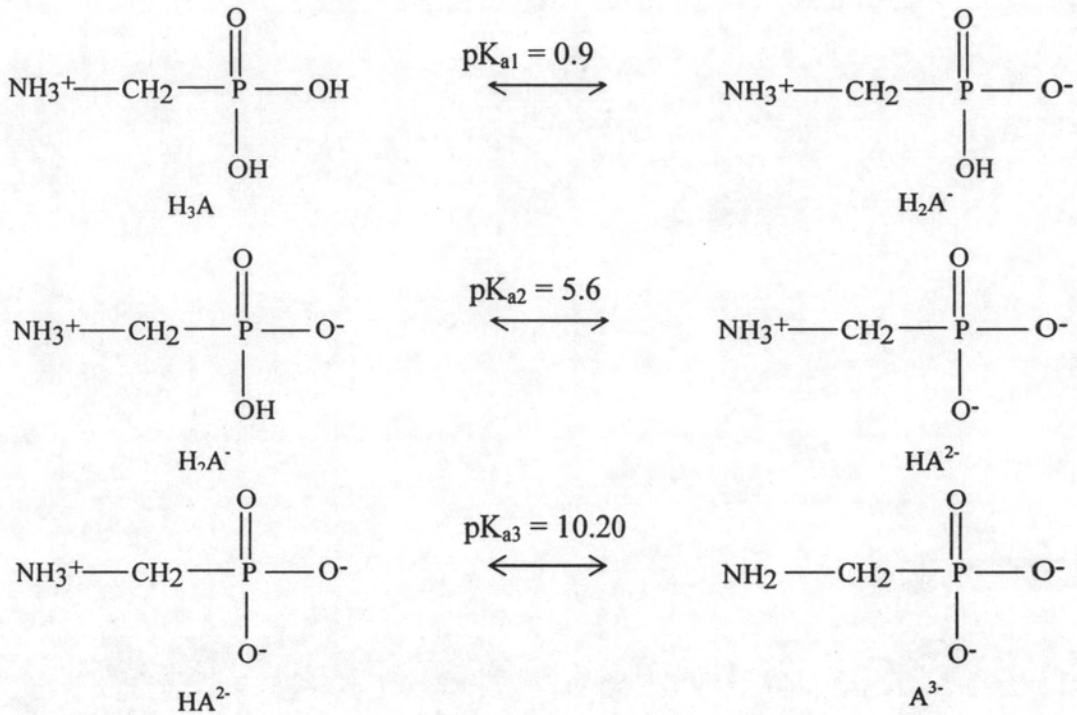

Figure 4.3 AMPA equilibria.

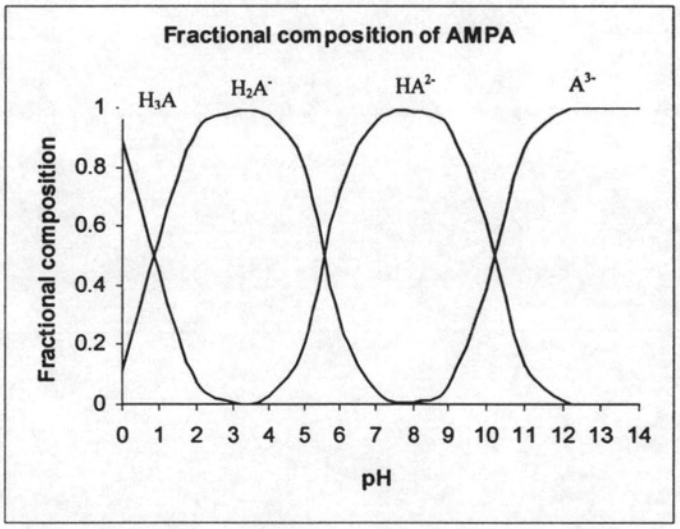

Figure 4.4 Fractional composition diagram of AMPA.

Fractional dissociation diagrams suggest that glyphosate and AMPA have potential to bear negative charges more than positive charge especially at higher pH. At this condition, suitable carrier should be positively charged. The literature review suggests that Aliquat 336 is the only carrier that can be effectively used for glyphosate and available commercially. At the beginning of this work, diethylhexyl phosphoric acid (DEHPA) was also evaluated as a carrier. DEHPA is a common ligand that dissolves well in organic solvent and can complex well with polar analyte especially cations. The donor solution was adjusted to pH 0 when both analytes are positively charged and should complex with DEHPA. However at this pH, DEHPA with pK_a value of 3.24 did not ionize. From our observation, both analytes were not detected by HPLC with post-column derivatization indicating the inefficient of DEHPA for the extraction of glyphosate and AMPA. Therefore, Aiquat 336 was selected as a carrier.

Aliquat 336 is a quaternary ammonium salt that has permanent positive charged independent of the bulk pH. Naturally, its long alkyl chains are hydrophobic but the quaternary ammonium cation is hydrophilic. These two functions allow Aliquat 336 to dissolve in both aqueous and polar organic solvents. We tested several types of organic solvents such as dodecane, dodecane modified with dodecanol, kerosene and di-n-hexyl ether. Dodecane was immiscible with Aliquat 336 therefore dodecanol was added to dodecane in order to enhance solubility of Aliquat 336 in dodecane. However, dodecanol is soluble in water and loss of carrier and extracting solvent was observed. Di-n-hexyl ether is a polar organic solvent immiscible with water and can dissolve in Aliquat 336. Comparison of organic solvents properties are shown in Figure 4.5. Although the EF of di-n-hexyl ether was lower than other solvents, it showed better reproducibility and was selected as the solvent of choice for this work.

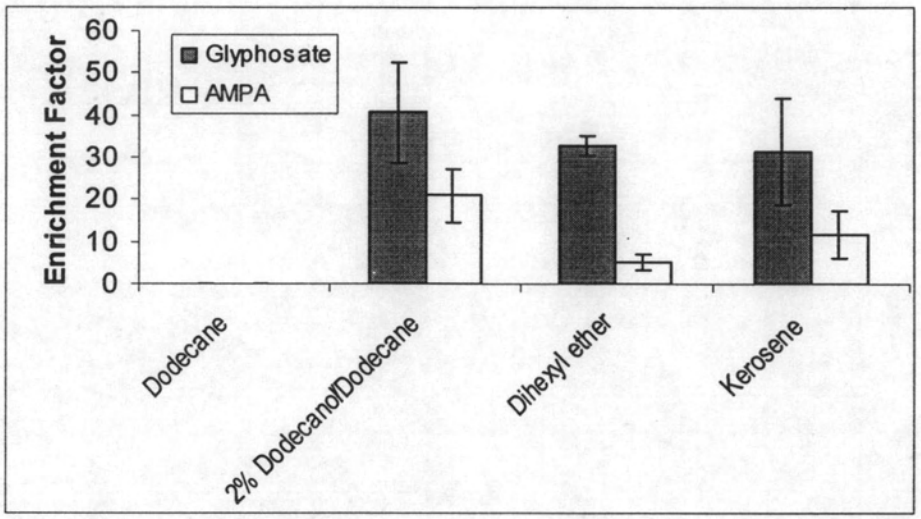

Figure 4.5 Result of organic solvent type variation. (extraction condition: the donor solution pH 11, 3.5 mL; the acceptor solution 1 M HCl, 30 µL; the membrane solution 0.2 M Aliquat 336; extraction time 30 minutes; immersion time 20 minutes)

4.2 Glyphosate and AMPA with Carrier-Mediated Transport using Aliquat 336

As described in section 2.2.2, glyphosate and AMPA can be considered as anionic. The transport mechanism of glyphosate and AMPA with Aliquat 336 is presented in Figure 4.6.

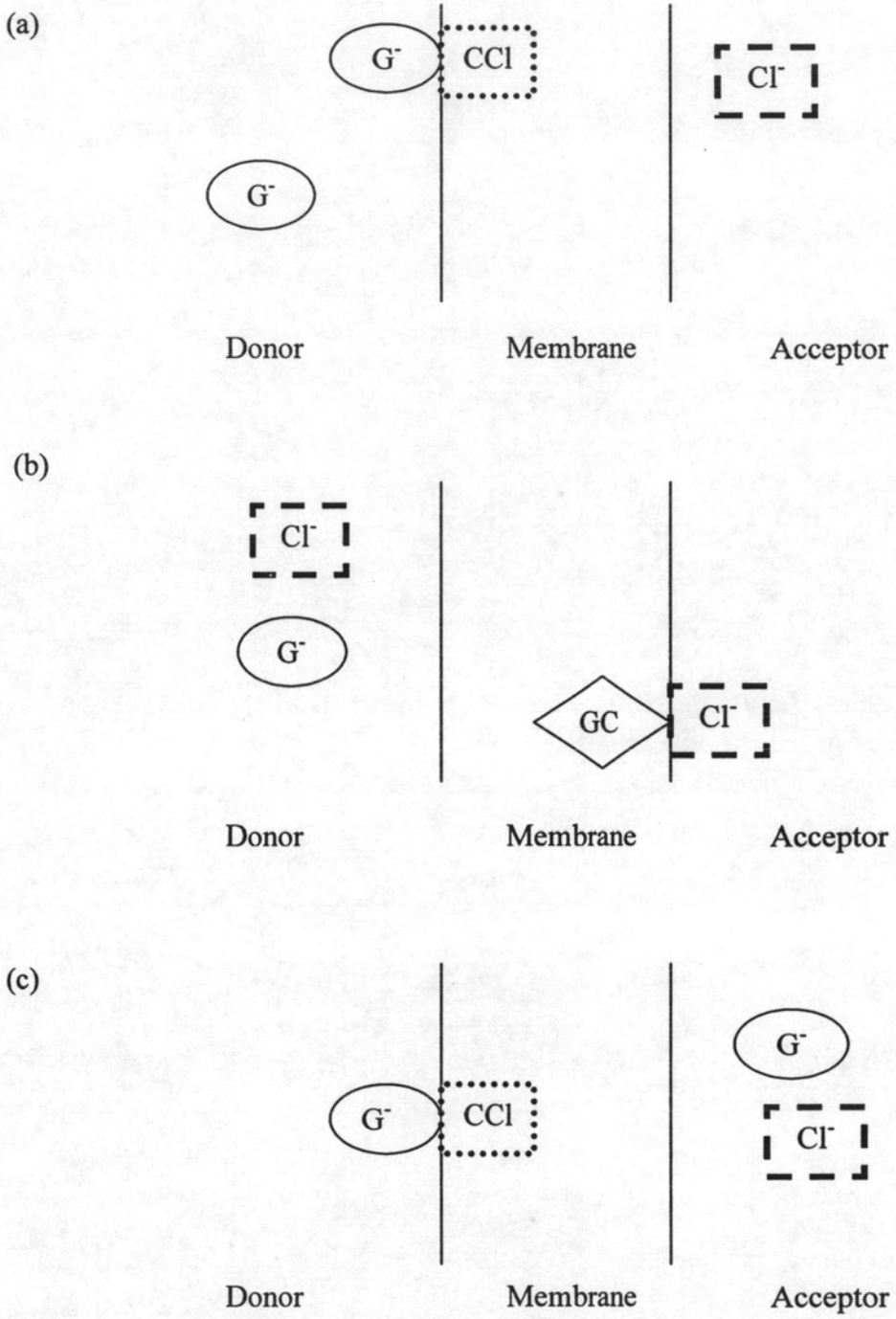

Figure 4.6 Carrier-mediated transport of glyphosate. (G^- = anionic analyte, CG = glyphosate-carrier complex, CCl = carrier, Cl^- = chloride ion)

The first step (a) is the partition of an anion analyte from bulk solution at donor solution-membrane interface where the analyte complexes with quaternary ammonium cation of Aliquat 336. This complex is diffused through the membrane

solution to the membrane-acceptor solution interface (b). Here, the interchange of analyte and chloride ion occurrence results in the release of anionic analyte into the acceptor solution. The carrier then diffuses back to the donor-membrane interface and binds with new analyte molecule continuously (c). At the same time, chloride ion is liberated into the donor solution in reverse order.

4.3 HF-LPME Optimization

In HF-LPME, parameters affecting extraction efficiency should be studied. The optimized procedure will provide the best sample preparation efficiency. As described in chapter 2 (section 2.4), the extraction efficiency can be described by enrichment factor (EF) which is the ratio of the analytes in the acceptor phase over the concentration in the donor phase. The parameters that influence this ratio were carefully evaluated. Each parameter was studied in three replicates. The extracts (acceptor solution) were directly injected into the HPLC with post-column derivatization.

4.3.1 Immersion Time

The first parameter to study was the immersion time which was the submerging time of hollow fiber membrane (HFM) in the organic solvent. The HFM was polypropylene that was hydrophobic. Therefore, it was immersed in hydrophobic organic solvent (di-n-hexyl ether mixing with Aliquat 336 as a carrier) that can seep through the membrane pores. Excess solvent remained in the lumen was eliminated by forcing air through the lumen from a 3-mL medical syringe.

Immersion times were varied at 5, 30, 60, 120 minutes and overnight. Table 4.1 and Figure 4.7 showed the EFs and the standard deviations caused by varying the immersion time. In general, longer immersion time provided better standard deviations because sufficient time was allowed for the solvent to completely fill the membrane pores resulting in greater reproducibility. Therefore, overnight immersion was selected because not only it can provide maximum reproducibility but the procedure is simple for routine analysis

where the HFM can be soaked overnight. The fiber was only used once and discarded, totally eliminating the carry-over effect.

Table 4.1 Average EFs and standard deviations of varying immersion time.

Immersion time (minutes)	Average EF ± SD (n=3)	
	Glyphosate	AMPA
5	291.87 ± 39.10	92.18 ± 3.54
30	281.44 ± 47.35	98.19 ± 11.37
60	271.44 ± 21.84	92.00 ± 4.24
120	287.90 ± 11.34	92.99 ± 5.77
Overnight	277.38 ± 11.30	98.30 ± 0.70

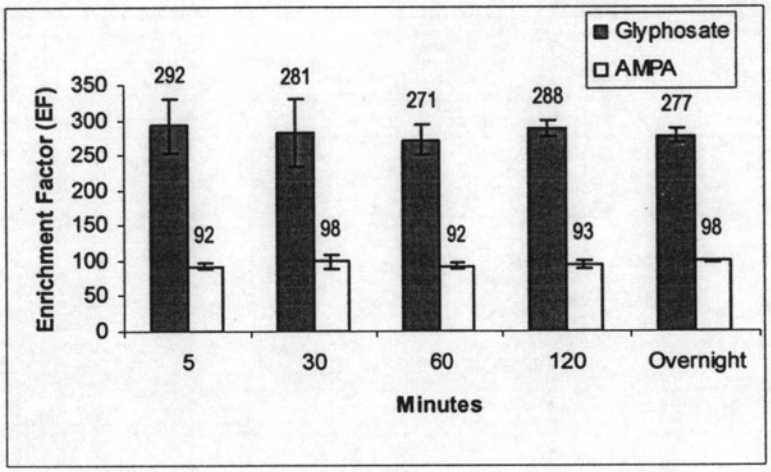

Figure 4.7 The influence of immersion time on enrichment factor.

This parameter was observed first because immersion time bares great influence on method reproducibility. Once method reliability is high and the results showed acceptable standard deviation, it is simpler to study the effects of other parameters.

4.3.2 Donor Solution pH

The donor solutions spiked with analytes was adjusted to pH 1.0, 3.0, 6.0, 8.0, 9.0, 10.0, 11.0 and 12.0 by hydrochloric acid and sodium hydroxide. Table 4.2 and Figure 4.8 showed the effects of donor solution pH on the EFs.

Table 4.2 Average EFs and standard deviations of varying donor solution pH.

Donor solution pH	Average EF ± SD (n=3)	
	Glyphosate	AMPA
1	0.00 ± 0.00	0.00 ± 0.00
3	0.00 ± 0.00	0.00 ± 0.00
6	27.01 ± 5.38	0.00 ± 0.00
8	363.70 ± 40.59	131.63 ± 14.84
9	408.50 ± 34.33	170.49 ± 9.25
10	593.20 ± 75.33	151.11 ± 19.18
11	281.14 ± 26.89	96.66 ± 8.50
12	100.51 ± 0.00	22.78 ± 0.00

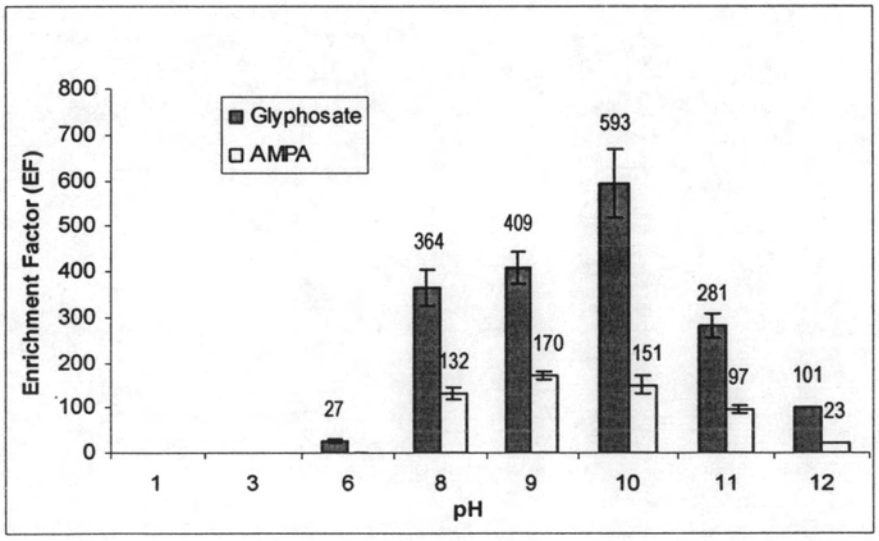

Figure 4.8 The influence of the donor solution pH on enrichment factor.

Figure 4.8 showed that when the pHs of the donor were above pH 8.0, the EFs increased hundred folds. These phenomena can be explained by the acid-base equilibria of polyprotic acid. Species of the polyprotic analytes are varied by pH of the bulk solution. Glyphosate and AMPA fractional diagrams are presented in section 4.1.

It can be concluded from the diagrams that when the solution pH is 8.0 and higher, the fraction of HA^{3-} and A^{4-} of glyphosate and HA^{2-} and A^{3-} of AMPA. These species preferred to complex with cationic-carrier such Aliquat 336 because they have more active anionic sites. Especially, at pH 10 and above, highly charged A^{3-} and A^{4-} species meanly presence resulted in exceptionally high EF. However, above pH 10.0, the donor solutions were turbided due to the loss of Aliquat 336. The solubility of Aliquat 336 increased at high pH. When the donor pH was above 10.0, Aliquat 336 seeped out of the membrane pore taking the organic solvent (di-n-hexyl ether) along. Di-n-hexyl ether is immiscible in water resulting in the observed cloudiness of the donor solution. Therefore modifying the donor pH can be used to simply improve the EFs of glyphosate and AMPA nearly 600 times indicating that the donor pH is a primary extraction parameter. The donor solution at pH 9.0 was selected as the optimum value that compromised for the maximum sensitivity of both glyphosate and AMPA.

4.3.3 Concentration of Aliquat 336

A carrier, Aliquat 336 (tricaprylylmethylammonium chloride), is a quaternary ammonium salt that can ionize to quaternary ammonium cation. The cationic carrier complexes with glyphosate and AMPA anions at the interface of donor solution-membrane interface. This complex diffuses through the organic solvent in the membrane pores to the acceptor-membrane interface where the analytes are discharged into acceptor solution. The cationic carrier is then recycled to complex with new molecules of glyphosate and AMPA. Therefore, its free form should be available for analytes transportation at all time and should have fast equilibrium system.

Membrane solvent was prepared by mixing Aliquat 336 and di-n-hexyl ether at varying compositions as shown in Table 3.2. Table 4.3 and Figure 4.9 showed influence of carrier concentration on EFs. EFs continued to increase at increasing Aliquat 336 concentration until peaked at 0.20 M. The drop in EFs and increasing standard deviation after 0.20 M were due to seepage of acceptor phase into aqueous media (donor) due to increasing solubility of Aliquat 336 at higher pH. The leaking of Aliquat 336 also resulted in the loss of the membrane solvent, di-n-hexyl ether, which could be clearly observed as turbid solution in the donor phase.

Table 4.3 Average EFs and standard deviations of varying Aliquat 336 concentration.

Aliquat 336 concentration (M)	Average EF ± SD (n=3)	
	Glyphosate	AMPA
0.05	23.64 ± 3.84	1.63 ± 0.84
0.10	103.97 ± 5.62	25.73 ± 7.38
0.15	235.33 ± 1.82	84.22 ± 6.77
0.20	378.14 ± 32.73	168.37 ± 11.76
0.25	362.08 ± 55.41	205.18 ± 19.94
0.30	338.63 ± 95.22	246.79 ± 83.50

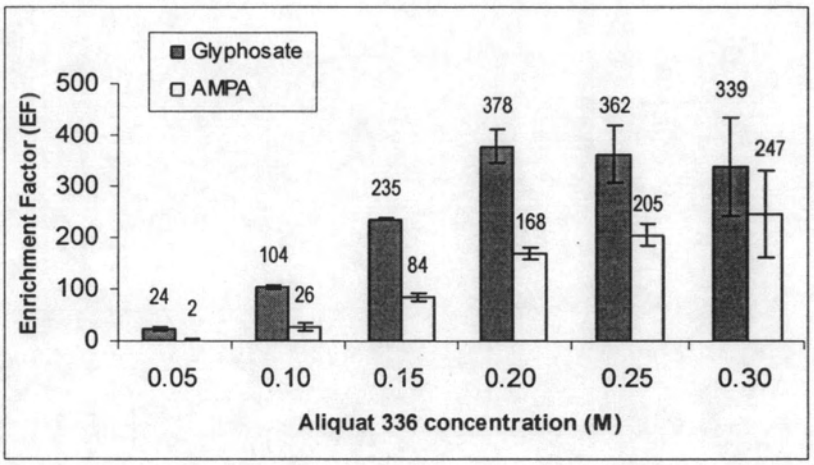

Figure 4.9 The influence of the Aliquat 336 concentration on enrichment factor.

4.3.4 Type of Acceptor Solution

From section 4.2, chloride ion in the acceptor phase characterizes as a counter-ion in analyte transportation. At the acceptor-membrane interface, the analyte-carrier complex should split and the analyte dissolves into the acceptor solution, leaving the carrier free to complex with another molecule of analyte at the donor-membrane interface. Therefore, suitable acceptor was one of the primary parameters observed. Formic acid, hydrochloric acid, sodium chloride, potassium chloride, and ammonium chloride in Milli-Q water were tested at 1.0 M concentration.

Table 4.4 and Figure 4.10 showed the EFs and standard deviations of several acceptor solutions. Only monovalent cation salts and acids were chosen due to concern about special cation-exchange glyphosate column. Formic acid provided no chloride counter-ion and therefore the EFs obtained were very low. Hydrochloric acid (1.0 M, pH=0), a strong acid, was completely dissociated and therefore provided high concentration of chloride for transport mechanism which resulted in higher EFs. Chloride salts provided the best EFs values because when pH is near zero (as for HCl), glyphosate and AMPA species existed in acid forms (H_4A and H_3A) and therefore the solution contained less charged species than using hydrochloric acid as an acceptor.

This limit the solubility of both analytes at the acceptor interface resulting in small EFs when use hydrochloric acid. No different among the EFs of chloride salts was observed so potassium chloride was chosen due to its better standard deviation. This could be because the K^+ counter ion in ion-exchange column used is compatible to existing K^+ in potassium chloride solution.

Table 4.4 Average EFs and standard deviations of varying acceptor solution type.

Acceptor solution type	pH	Counter ion	Average EF ± SD (n=3)	
			Glyphosate	AMPA
Formic acid	2	-	27.05 ± 5.73	0.00 ± 0.00
Hydrochloric acid	0	Cl^-	104.42 ± 17.22	75.48 ± 11.05
Sodium chloride	7	Cl^-	375.48 ± 31.65	151.49 ± 12.27
Potassium chloride	7	Cl^-	393.85 ± 19.83	165.88 ± 14.39
Ammonium chloride	7	Cl^-	352.90 ± 84.60	156.55 ± 71.72

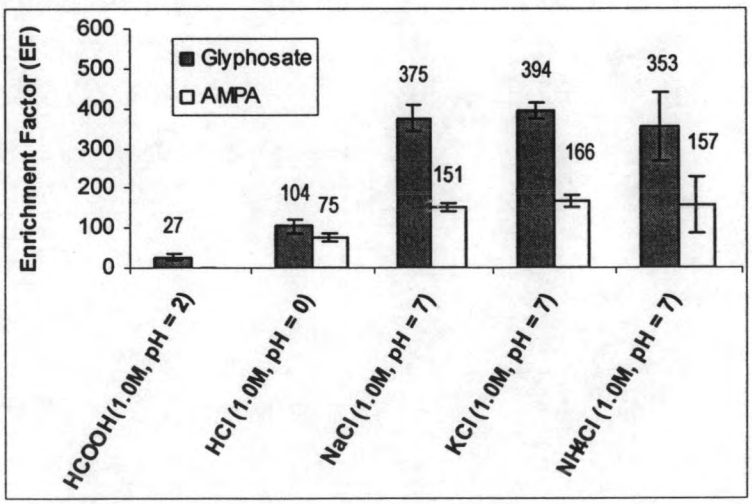

Figure 4.10 The influence of the acceptor solution type on enrichment factor.

4.3.5 Acceptor Solution Concentration

Because chloride ion should be available for transport across the interface into the membrane pores, its' concentration in the acceptor solution should be sufficient. Potassium chloride solution at 0.25, 0.50, 0.75, 1.0, 1.50, and 2.0 M were tested.

Increasing of EFs along with increasing chloride concentration was observed until 1.0 M afterwhich the EFs were nearly constant (Figure 4.11). Table 4.5 showed EFs and standard deviations of this step. This can be explained that at pH 7, glyphosate and AMPA bear negative charges. And therefore they competed with chloride counter-ion in the acceptor and can be back extracted to the donor. Another reason is that the two analytes concentrations reached the limit of solubility at this chloride concentration where no more analytes could further dissolve resulting in stable EF at higher chloride ion concentration.

Table 4.5 Average EFs and standard deviations of varying concentration of acceptor solution.

Acceptor solution concentration (M)	Average EF $\pm$ SD (n=3)	
	Glyphosate	AMPA
0.25	99.25 $\pm$ 28.60	87.98 $\pm$ 17.90
0.50	224.17 $\pm$ 7.65	109.08 $\pm$ 20.85
0.75	294.58 $\pm$ 24.56	119.70 $\pm$ 15.76
1.00	417.74 $\pm$ 22.98	152.43 $\pm$ 12.82
1.50	408.58 $\pm$ 18.87	139.30 $\pm$ 16.29
2.00	413.50 $\pm$ 19.09	116.02 $\pm$ 15.28

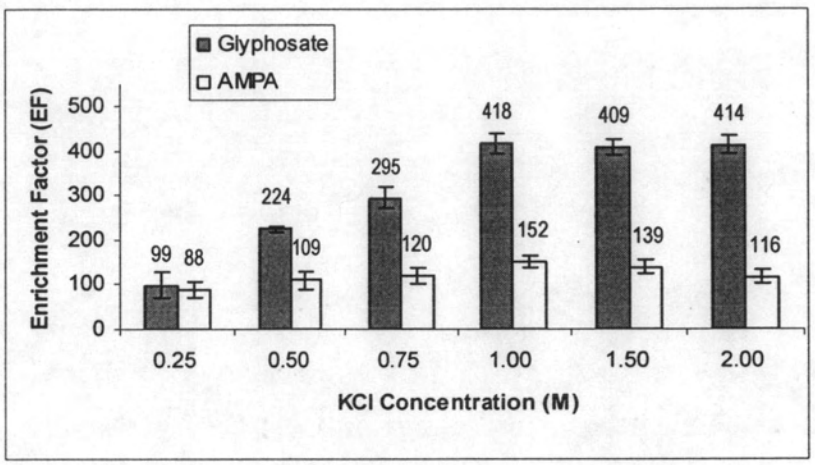

Figure 4.11 The influence of the acceptor solution concentration on enrichment factor.

4.3.6 Acceptor Solution Volume

In equation 6 (section 2.4), EF is depended on the phase ratio which is the ratio of donor volume and acceptor volume.

$$EF = EE\frac{V_D}{V_A} \qquad \text{(Eq. 6)}$$

If we reduced the volume of the acceptor solution, EF will increase. The HFM lumen contains the acceptor solution only in microliters scale capacity. Segments of hollow fiber were cut into 4.0, 8.0, 12.0, 20.0, 31.0 and 37.0 cm and filled with acceptor solution at 10.0, 20.0, 30.0, 50.0, 80.0 and 100.0 μL, respectively (Table 3.3).

The EFs in Table 4.6 and Figure 4.12 obeyed equation (6) until 10.0 μL. The optimum EFs was observed at 20.0 μL. This can be explained by Fick's law that stated:

$$J = \frac{DA(C1 - C2)}{L} \qquad \text{(Eq. 11)}$$

where J is diffusion rate. D is diffusion coefficient. A is surface area. L is wall thickness of HFM and C1-C2 is concentration gradient.

Surface area of HFM is directly related to mass flux. The short HFM has smaller surface area than the longer one. For 10.0 and 20.0 μL acceptor solution volume, the increasing in surface area of 20.0 μL acceptor volume was more effective than the decreasing phase ratio at 10.0 μL acceptor volume.

Table 4.6 Average EFs and standard deviations of varying acceptor solution volume.

Acceptor solution volume (μL)	Average EF ± SD (n=3)	
	Glyphosate	AMPA
10.0	339.47 ± 26.88	34.96 ± 6.79
20.0	444.89 ± 21.77	184.52 ± 15.30
30.0	401.45 ± 23.32	179.70 ± 15.76
50.0	198.53 ± 16.47	186.30 ± 26.86
80.0	126.57 ± 8.51	163.35 ± 22.22
100.0	100.06 ± 3.51	160.09 ± 11.62

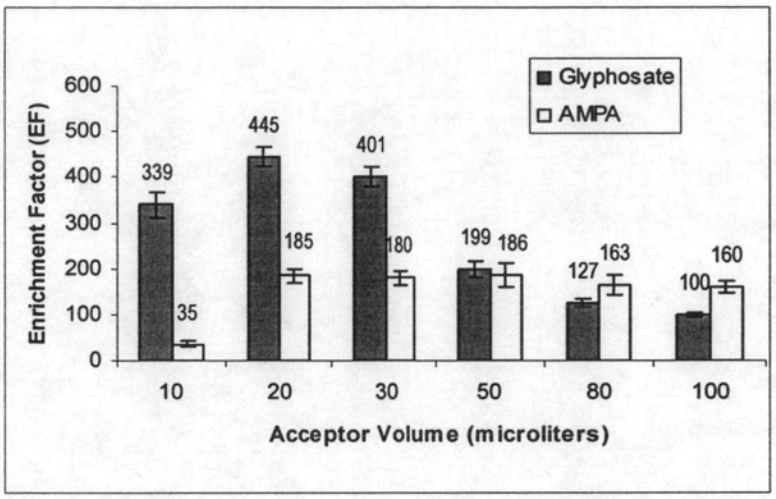

Figure 4.12 The influence of the acceptor solution volume on enrichment factor.

4.3.7 Donor Solution Volume

It is expected from equation (6) that high EFs can be obtained at large donor volume. Therefore, this volume was optimized at 3.5 to 120.0 mL. Table 4.7 showed EFs of donor solution optimization. Figure 4.13 showed increasing EFs at increasing donor volume. However, the standard deviations also increased due to leaching of Aliquat 336 into the donor solution. A 20.0 mL donor solution was selected to compromise for both the extraction efficiency and smaller sample volume.

Table 4.7 Average EFs and standard deviations of varying donor solution volume.

Donor solution volume (mL)	Average EF ± SD (n=3)	
	Glyphosate	AMPA
3.5	111.22 ± 9.75	117.61 ± 1.24
10	149.57 ± 15.55	95.84 ± 9.92
20	462.32 ± 38.17	177.11 ± 31.29
40	1160.47 ± 323.59	191.41 ± 110.78
60	869.71 ± 242.61	124.00 ± 24.95
120	506.02 ± 292.17	45.74 ± 26.41

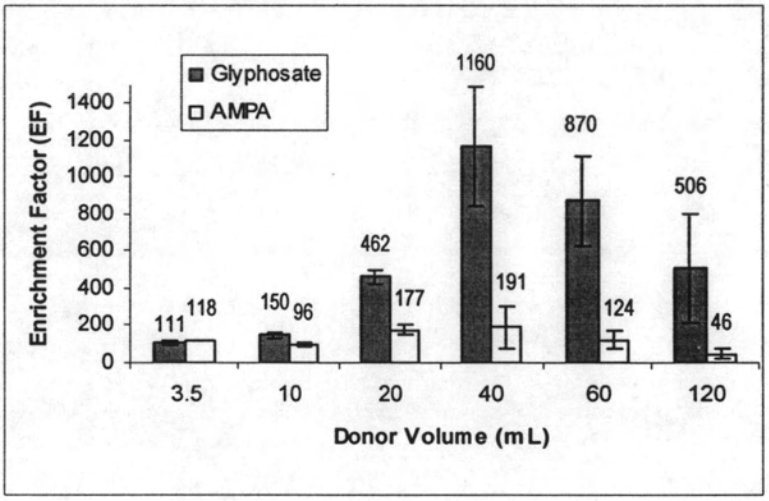

Figure 4.13 The influence of the donor solution volume on enrichment factor.

4.3.8 Agitation

To increase the mass transfer coefficient, agitation by vortex was performed at different speed. A magnetic stir bar was not used to minimize carry-over and cross contamination. The system was vortexed at ambient temperature at arbitrary units that were increased from 1 to 8. No agitation by other mean was performed.

Table 4.8 and Figure 4.14 showed that optimum and good standard deviations were obtained when vortexed at 3 arbitrary unit. At higher unit, standard deviations were increased noticibly and turbid solution was also observed. We expected that excessive turbulent flow of donor solution at high speed destroyed the supported liquid membrane system. Consequently, the vortex unit set at 3 was selected.

Table 4.8 Average EFs and standard deviations of varying agitation.

Agitation	Average EF ± SD (n=3)	
	Glyphosate	AMPA
No agitation	99.51 ± 15.74	106.20 ± 9.06
Vortex 1	533.79 ± 83.93	120.92 ± 17.07
Vortex 2	765.27 ± 64.68	153.03 ± 8.92
Vortex 3	796.49 ± 22.80	160.44 ± 8.70
Vortex 4	737.94 ± 49.29	158.06 ± 3.06
Vortex 5	484.72 ± 64.06	160.85 ± 29.79
Vortex 6	354.69 ± 160.99	87.50 ± 21.46
Vortex 7	371.65 ± 160.42	93.56 ± 38.46
Vortex 8	341.02 ± 185.92	94.87 ± 29.32

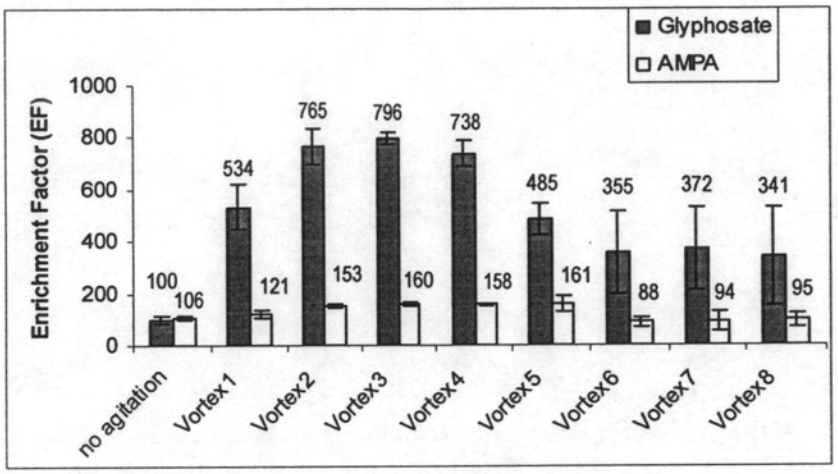

Figure 4.14 The influence of the agitation on enrichment factor.

4.3.9 Extraction Time

Because the three-phase liquid phase microextraction with hollow fiber membrane (HF-LPME) is a non-exhaustive extraction method, maximum EFs is reached at equilibrium. Equilibrium time was determined at 10, 20, 30, 45, 60 and 90 minutes. Table 4.9 and Figure 4.15 showed increasing EFs at longer extraction time and equilibration were observed at around 60 minutes.

Table 4.9 Average EFs and standard deviation of varying extraction time.

Extraction time (minutes)	Average EF ± SD (n=3)	
	Glyphosate	AMPA
10	124.15 ± 19.04	48.01 ± 2.43
20	536.19 ± 72.26	78.56 ± 13.44
30	590.94 ± 50.73	168.30 ± 17.99
45	803.75 ± 43.01	166.73 ± 32.39
60	832.72 ± 12.02	163.35 ± 19.47
90	876.85 ± 64.82	174.44 ± 30.11

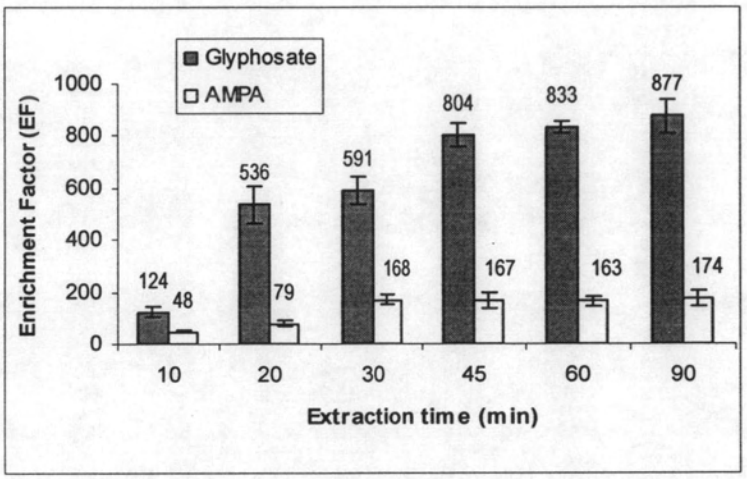

Figure 4.15 The influence of the extraction time on enrichment factor.

The optimum HF-LPME condition was concluded in Table 4.10. An 8-cm HFM was immersed in 0.20 M Aliquat 336 in di-n-hexyl ether overnight, then the lumen was filled with 20.0-μL of 1.0 M potassium chloride acceptor solution. It was used to extract 20.0-mL donor solution of pH 9.0 for 60 minutes. The donor solution was vortexed at unit 3. After extraction, the acceptor solution was directly injected into HPLC with post-column derivatization system.

Table 4.10 Optimum extraction condition of HF-LPME.

Parameters	Extraction condition
Donor pH	9.0
Donor volume	20.0 mL
Acceptor	1.0 M KCl
Acceptor volume	20.0 μL
Aliquat 336 concentration	0.20 M
Immersion time	Overnight
Agitation	Vortex 3
Extraction time	60 min

The optimum condition was tested to extract 10 replications of spiked standard solution at 5 μg/L. The average EFs of glyphosate and AMPA were 814 and 141, respectively. EF of AMPA was lower than glyphosate. In chromatogram (Figure 2.11), glyphosate is eluted faster than AMPA because AMPA molecules bonded stronger to sulfonate groups than glyphosate. In the similar way, AMPA bonded to carrier more tightly which resulted in less liberated molecules in the acceptor solution and thus lower EF was observed.

4.4 Method Validation

Method validation is the process of proving that our proposed HF-LPME procedure is acceptable in analytical perspective. This process is important for routine works.

4.4.1 The Linear Dynamic Range

To determine the useful working range for the developed HF-LPME procedure for glyphosate and its metabolite AMPA in water samples, their linear dynamic ranges must be constructed. Linear dynamic range is the concentration interval that acceptable linearity can be obtained. MQLs was

determined to be up to 1,000 μg/L for glyphosate and 605 μg/L for AMPA where the instrument responds departed from linearity. The developed HF-LPME procedure was tested using 2 replicates of spiked reagent water. Intercepts, slopes, and correlation coefficients of the two analytes were presented in Table 4.11. The results showed very good correlation coefficients for both glyphosate and AMPA at above 0.99.

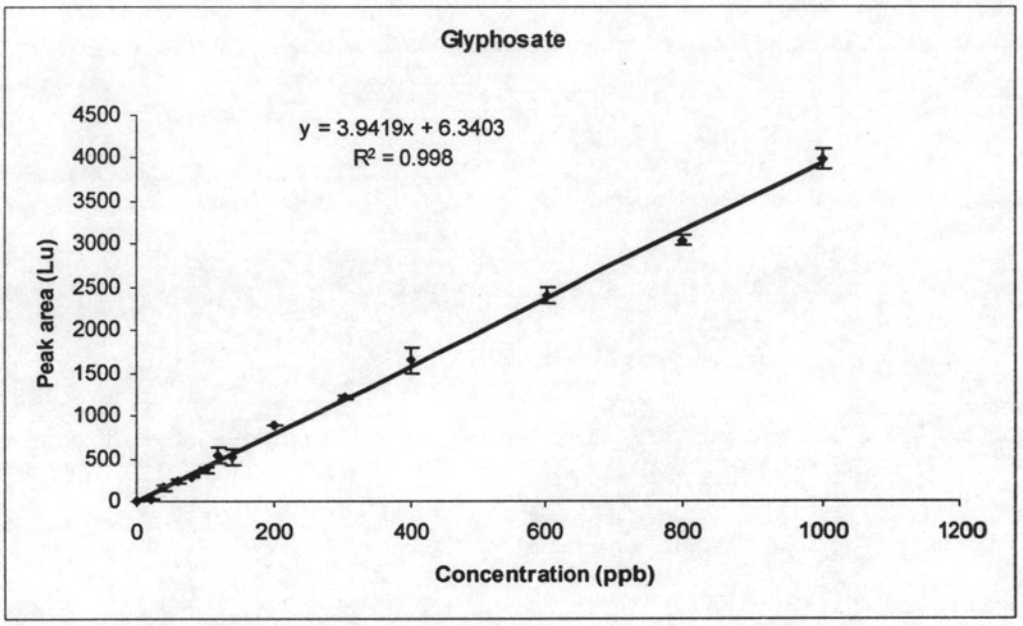

Figure 4.16 Linear dynamic range of glyphosate.

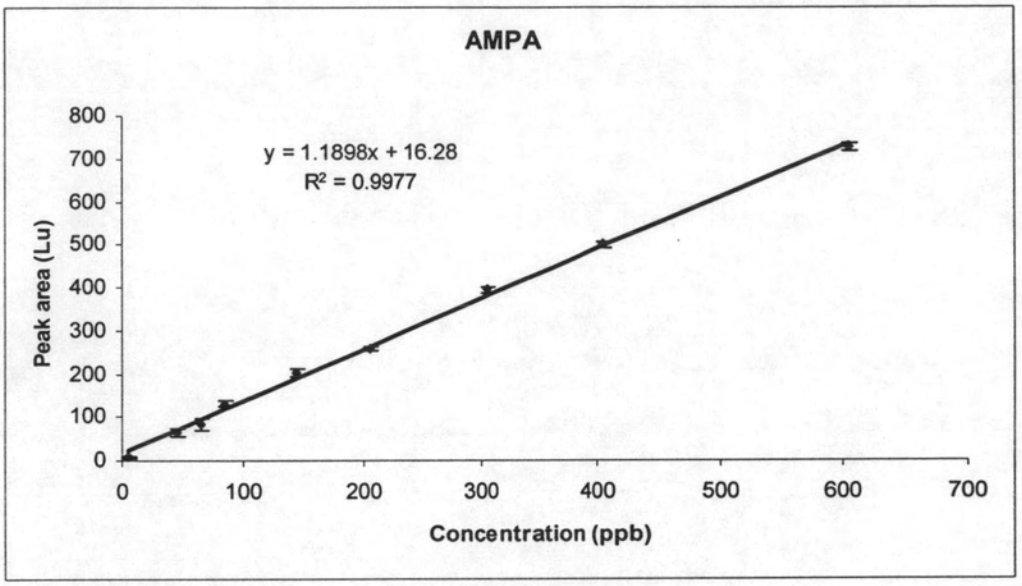

Figure 4.17 Linear dynamic range of AMPA.

Table 4.11 Intercept, slope and correlation coefficient (R^2) of glyphosate and AMPA.

	Glyphosate	AMPA
y-Intercept	6.3403	16.28
Slope	3.9419	1.1898
Correlation coefficient	0.9980	0.9977

4.4.2 The Method Detection Limits (MDLs) and Quantitation Limits (MQLs)

In this section, the developed HF-LPME procedure was tested and validated using 10 replicates of spiked reagent water. After extraction, the acceptor solutions were analyzed by HPLC with post-column derivatization. The method quantitation limit (MQL) was determined as the concentration of analytes that give the peak signal at signal-to-noise-ratio equal to 10. Method detection limit (MDL) was determined as the concentration of the analytes that give the peak signal at signal-to-noise-ratio equal to 3. The MDL and MQL

values of both analytes are summarized in Table 4.12. The MDL was compared to the recommended value of the EPA standard method 547.

Table 4.12 Method detection limits (MDLs) and method quantitation limits (MQLs) of glyphosate and AMPA (n=10).

Compound	MDL (μg/L)	MQL (μg/L)	MDL of EPA method 547 (μg/L)
Glyphosate	0.32 ± 0.08	1.03 ± 0.20	6.0
AMPA	1.50 ± 0.19	5.01 ± 0.31	-[a]

[a] No report.

The results show that, the MDL and MQL of glyphosate are lower than AMPA's because optimum EF of glyphosate was higher than AMPA's. Glyphosate exhibits greater sensitivity for HF-LPME than AMPA. MDLs and MQLs stabilities are also depended on the freshness sodium hypochlorite solution of which degraded by time. From our observation, fresh oxidizing reagent should be prepared daily and the post-column instrument calibrate with this freshly prepared solution prior actual analysis.

The method detection limit (MDL) obtained for glyphosate was lower than the requirement of the EPA standard method. Therefore injection volume of 5-μL is sufficient comparing to the required 200-μL injection volume of the standard method. Both glyphosate's and AMPA's MQLs of the developed procedure are lower than the recommended maximum contaminant level for water at 0.7 mg/L.

4.4.3 The Precision

The precision of an analytical method can be measured by the amount of scattering in the data of multiple analyses of a homogeneous sample. To be meaningful, the precision study must be performed using the exact sample and standard preparation procedures that will be used in the final method.

Generally, the precision was presented as the percent relative standard deviation (%RSD) of repeated analyses. The data presented here are intra-assay precision that were obtained by repeated analysis, in one laboratory on the same day. Aliquots of a homogeneous sample, each of which has been independently prepared according to the developed HF-LPME procedure were analysed in 10 replications. The acceptor solutions were analyzed by the HPLC procedure with post-column derivatization. The analyses were performed at MQLs and 5-fold MQLs concentrations. The obtained %RSD values were compared to the values calculated by Horwitz equation (40).

$$RSD_r = 0.67 * 2^{(1-0.5\log C)} \qquad \text{(Eq. 12)}$$

where RSD_r is relative standard deviation and C is mass fraction (for 100%, C = 1.00; for 1 mg/L, C = 0.000001; for 1 µg/L, C = 0.000000001)

.

Table 4.13 Relative standard deviation of glyphosate and AMPA spiked at MQLs and 5-fold MQLs concentrations (n=10) compared to values calculated by Horwitz equation.

	Concentration (µg/L)	Relative standard deviation (%RSD)		
		Glyphosate	AMPA	Horwitz value
MQL	1	12.68	-	30.32
	5	-	7.33	23.80
5-MQL	5	11.86	-	23.80
	25	-	10.34	18.68

Table 4.13 showed %RSD of HF-LPME method comparing with %RSD calculated by Horwitz equation. The calculated Horwitz values at MQL and 5-MQL levels ranged from 23.80-30.32 % and 18.68-23.80 %, respectively. The experimentally determined %RSD values of glyphosate are 12.68 % at

MQL and 11.86 % at 5-MQL. The experimentally determined %RSD values of AMPA are 7.33 % at MQL and 10.34 % at 5-MQL. Both experimentally obtained %RSD values are all below the %RSD calculated by Horwitz equation. The results indicate that the method is sufficiently precise at the concentration level of analytes being measured.

4.4.4 The Accuracy

The accuracy of a method is the closeness of the measured values to the true value of the sample. The approach is based on the recovery of known amounts of analyte spiked into the sample matrix. In this method, percent recovery was used to compare the recovered concentrations to the actual spiked quantity in reagent water. Glyphosate was spiked at 3 μg/L and AMPA was spiked at 8 μg/L. The procedure was performed in 10 replications. The results of method accuracy presented by % recovery of both analytes are shown in Figure 4.14.

Table 4.14 Percent recovery of glyphosate and AMPA (n=10).

Compound	Recovery (%)	RSD (%)
Glyphosate	100.06 ± 0.54	18.21
AMPA	107.45 ± 1.47	17.07

The experimental accuracies can be considered using percent recovery method as published in the AOAC manual for Peer Verified Methods Program (41). The data obtained met the AOAC's requirement that estimated the range of recovery at 46-118 % for 3 μg/L level and 56-116 % for 8 μg/L level. Experimental recoveries of spiked sample were measured to be 100.06 % for glyphosate and 107.45 % for AMPA. Both experimental recoveries were within the acceptable range of the AOAC manual for Peer Verified Methods Program. The experimental results indicated that the developed HF-LPME provided sufficient accuracy for the analysis of glyphosate and AMPA in water.

4.5 Testing Result of the HF-LPME Method Applied for the Determination of Glyphosate and AMPA in Ground Water Samples.

In order to evaluate the applicability of this HF-LPME method for the determination of glyphosate and AMPA in natural water, spiked ground water samples were subjected for analysis under the optimum condition followed by the detection by HPLC-post column derivatization. Samples of ground water collected from 4 sites in Thailand were tested for the presence of glyphosate and AMPA. The samples were extracted by the developed HF-LPME method and analyzed by HPLC with post-column derivatization. Neither glyphosate nor AMPA were detected in these samples. Therefore, these samples were spiked at 1 μg/L glyphosate and 5 μg/L AMPA standard solutions (MQL concentrations) and extracted following the developed procedure. The sample preparation step and analysis were performed in duplicate.

Table 4.15 Percent recovery of spiked ground water samples (n=8).

	Recovery (%)		RSD (%)	
Compound	HF-LPME	AOAC	HF-LPME	Horwitz value
Glyphosate	73.4-116.9	40-120	17.68	30.32
AMPA	60.8-102.5	50-117	15.02	23.80

The results are reported in Table 4.15. When compare to the requirement of the AOAC Manual for Peer Verified method, at MQLs, which allows 40-120 % range for glyphosate and 50-117 % range for AMPA. The experimental values were from 73.4-116.9 % for glyphosate and 60.8-102.5 % for AMPA which fell within the acceptable ranges at MQL.

From Horwitz calculation, the analytes at MQLs should vary no more than 30.32 % for glyphosate and 23.80 % for AMPA. The experimental values were 17.68 % for glyphosate and 15.02 % for AMPA that were almost half the Horwitz indicating excellent recovery in spiked ground water samples.

Both %relative recovery and %RSD are within the acceptable range published in AOAC manual for peer verified method and calculated by Horwitz equation. It is indicates that HF-LPME method has good sensitivity and can be used for natural water samples.

Figure 4.18 is the chromatogram of direct injection and Figure 4.19 is the chromatogram after HF-LPME enrichment of the same sample. The chromatograms clearly indicate the powerful enrichment power of the developed HF-LPME for glyphosate and AMPA.

Figure 4.18 The chromatogram of MQL level spiked ground water sample.

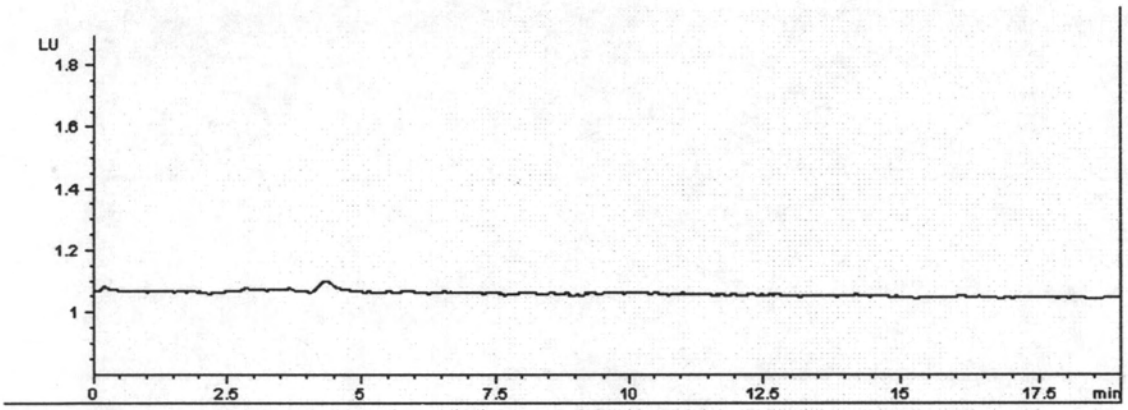

Figure 4.19 The chromatogram of MQL level spiked ground water sample extracted by HF-LPME.