Chapter 3

Results and discussions

Trace analysis of phthalate and adipate esters in packaged food was carried out by Gas-Liquid Chromatography or GLC where the mobile phase is gas and the stationary phase is liquid. In this thesis, a 30 m × 0.25 mm I.D., 0.25 μm film thickness fused silica HP-5MS capillary column was used and helium gas was used as the mobile phase. The HP-5MS capillary column is a processed commercial stationary phase from Hewlett Packgard, USA. Chemical composition of HP-5MS consists of 5% phenyl and 95% dimethylpolysiloxane. The flame-ionization detector was used for phthalate and adipate esters detection. Chromatographic conditions were optimized to obtain the best GLC performance.

3.1 Optimization of GC-FID conditions for phthalate and adipate esters

3.1.1 Carrier gas flow rate

The carrier gas (helium, He) flow rate was optimized by considering the relationship between the height equivalent to a theoretical plate (HETP) and the carrier gas flow rate. The HETP can be determined from the van Deemter plot (Figure 10) and calculated by the van Deemter equation that expresses the extent to which a component band spreads as it passes through the column in terms of physical constants and the velocity of the mobile phase as shown in Equation (1) (Grob, 2004).

$$\mathrm{HETP}\,(H) = A + \frac{B}{u} + Cu \qquad (1)$$

Where A is eddy diffusion term, a constant that accounts for the effects of "eddy" diffusion in the column

B is longitudinal or ordinary diffusion term, a constant that accounts for the effects of molecular diffusion of the vapor in the direction of the column axis.
C is resistance to mass transfer term, a constant propotional to the resistance of the column packing to mass transfer of solute through it.
u is the average linear velocity of carrier gas (mobile phase).

From this equation, the first term, eddy diffusion (A term) accounts for the geometry of the packing. This term describes the change in pathway and velocity of solute molecules in reference to the zone center. Therefore, when a sample migrates down the column, each molecule has different paths and each path is of a different length. Some molecules take the longer paths and other take the shorter paths. There are also variations in the velocities of the mobile phase within these pathways. The overall result is that some molecules lag behind the centre of the zone, whereas others move ahead of the zone. Therefore, the eddy diffusion process results in flow along randomly spaced variable-size particles in the column. Eddy diffusion term was quantified from the equation, $A = 2\lambda d_p$ where λ is a dimensionless constant characteristic of packing, and dp is the particle diameter of the packing. The contribution of the $2\lambda d_p$ term can be decreased by reducing the particle sized. As the particle size becomes smaller. The eddy diffusion shows the effect of H with changes in linear gas velocity. Equation (1) represents a hyperbola that has a minimum at velocity $u = (B/C)^{1/2}$ and a minimum H value (H_{min}) at $A + 2(BC)^{1/2}$.

The second term, $\frac{B}{u}$, describes the longitudinal diffusion term, $B = 2\gamma D_g/u$, D_g is the diffusion coefficients of the component in gas phase. This term is a measure of the effect of molecular diffusion on zone spreading. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion. The optimum velocity is greatest for column packed with small particles and for mobile phase in which the solutes have a high diffusivity *i.e.*, gases of low density, *e.g.*, hydrogen or helium.

However, the major contributing factor contributing to band broadening is the C term, which is the nonequlilibrium or resistance to mass transfer.

$C = (8/\pi^2)(k/(1+k)^2)(d_f^2/D_l)$ where k is retention factor (capacity factor), d_f is effective film thickness of liquid phase and D_l is diffusivity of solute in liquid phase. An obvious way of reducing this term is to reduce the liquid film thickness. This causes a reduction in k and an increase in the term $k/(1+k)^2$. The k term is temperature dependent, so we can increase k and decrease $k/(1+k)^2$ by lowering the temperature. Lowering the temperature increases viscosity and thus decrease D_l. Therefore, the effects of the factor $k/(1+k)^2$ and $1/D_l$ counteract each other. This term can be represented as the composite of the resistance to mass transfer in the mobile phase C_g and that in the stationary phase C_l: $C = C_g + C_l$. The C term accounts for resistance to mass transfer in the liquid phase (Grob, 2004).

From equation (1), at low u, the B term is large, but quickly diminishes with increasing u and C_g to a lesser extent, C_l, then dominate. The smallest value of H is $H_{\min}$, at with u is optimum, u_{opt}. The greater u_{opt}, the faster a sample can be analyzed (Baugh, 1993).

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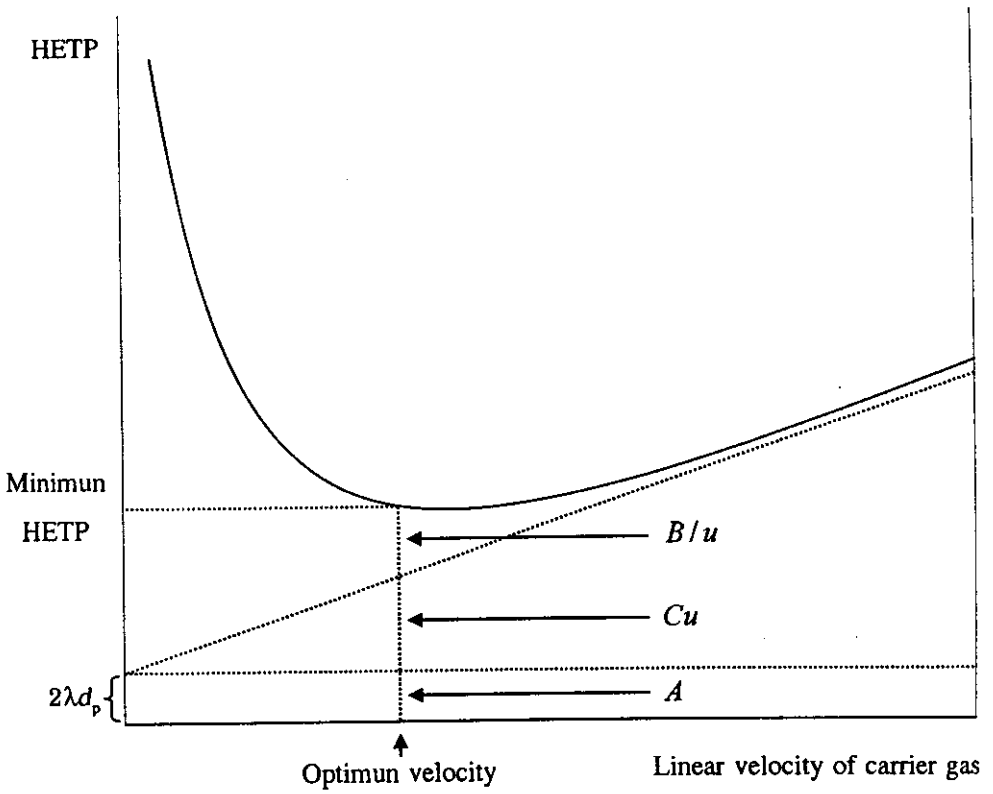

Figure 10 The van Deemter plot

In this research, a 30 m × 0.25 mm I.D., narrow bore wall-coated open tubular (WCOT) column or capillary column was used for the analysis. A capillary column is a fused-silica tube of very small internal diameter (generally between 0.20-0.53 mm). The inner surface of a capillary column is coated with a thin layer of stationary phase so it is still possible for the solute molecules to come in contact with the inner walls of the tubing. Most capillary column stationary phases are cross-linked and covalently bonded to the fused-silica surface. The amount of stationary phase in a capillary column is denoted by its film thickness, which is typically 0.1- 5.0 μm. Compound retention is proportional to film thickness in capillary columns, retention increases as the film thickness increases, and it decreases as the film thickness decreases (Michèle, 2003). In this column a liquid phase is coated on fused silica wall with no packing material, therefore, the A term (eddy diffusion) is nonexistent because there is only one flow path and no packing material. The resistance to mass transfer term C has the greatest effect on band broadening, and its effect in capillary

columns is controlled by the mass transfer in the gas phase C_g. Thus, Equation (1) takes a different form for capillary columns, and this known as the Golay equation (Grob, 2004) in Equation (2).

$$\text{HETP}\ (H) = B/u + C_g u \qquad (2)$$

The above equation showed that HETP is proportional to the flow rate of carrier gas (u). It is also known that an optimum carrier gas flow rate will give an optimum column resolution with the narrowest HETP (Grob, 2004)

In the practice, the terms of A, B and C in the equation, are difficult to obtain. However, the plate theory assumes that the column is divided into a number of zones called *theoretical plates* (N). The zone thickness or height equivalent to a theoretical plate (HETP) is determined by assuming that there is perfect equilibrium between the gas and liquid phases within each plate. The indication of column efficiency in the term of HETP (or H) is determined by Equation (3).

$$\text{HETP} = \frac{L}{N} \qquad (3)$$

Where L is length of column in centimeters

N is the number of theoretical plates

The plate number, N of a column can be calculated from Equation (4).

$$N = 16\left(\frac{t_R}{w}\right)^2 \qquad (4)$$

Where t_R is the retention time of the peak

w is the base peak width

If a width at half height ($w_{1/2}$) was used instead of a width (w) at the base, the plate number could be calculated by Equation (5) (Tibor and Esther, 1999) (Figure 11).

$$N = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2 \qquad (5)$$

Where $w_{1/2}$ is peak width at half height

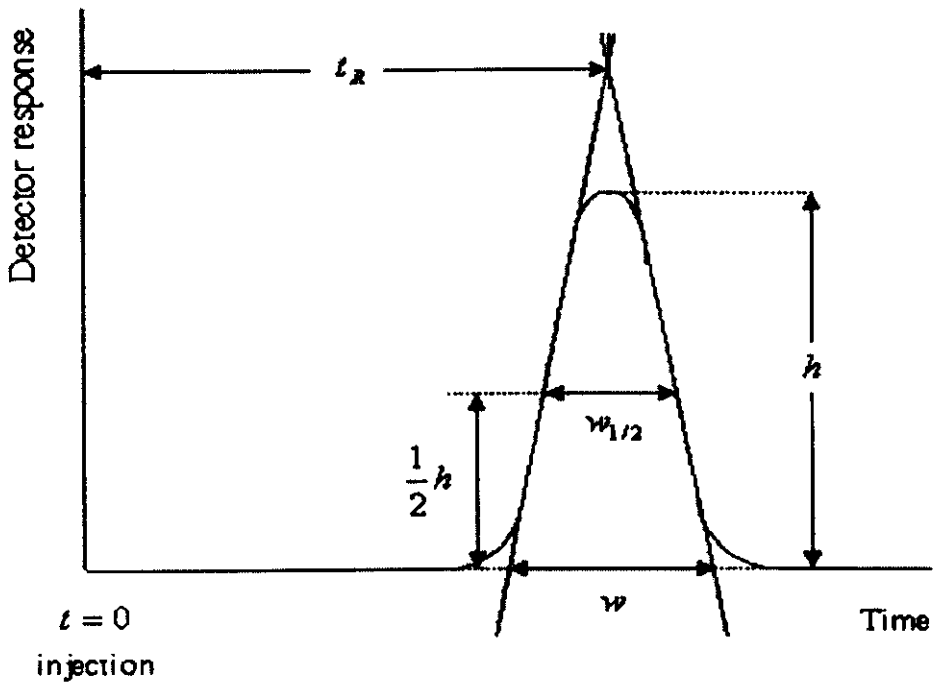

Figure 11 Measurement used in calculating total theoretical plates

Since a capillary column was used in this research, sharp peaks were obtained. It is difficult to determine the base peak width. Thus, the number of theoretical plates N was calculated directly from the value obtained from a chromatogram as shown in the Figure 11 by the relationship

$$N = 2\pi(t_R h / A)^2 \qquad (6)$$

Where t_R is the retention time, h is intergrated peak height and A is integrated peak area (Grob, 2004).

N was calculated from equation (6), and substituted in equation (3), with a known L term, column length, to obtain HETP. The HETP and the carrier gas flow rates (Table 9) were plotted as shown in Figure 13. From the van Deemter plot the

optimum flow rate of 1.2 mL min^{-1} was obtained at the narrowest HETP, which provided the highest column efficiency.

Table 9 The Height Equivalent to a Theoretical Plate (HETP) of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various carrier gas flow rates

Flow rate ($mL\ min^{-1}$)	**HETP×10^{-2} (cm)***	
	DEHP	**DEHA**
0.8	1.73	1.63
1.0	1.52	1.54
1.2	1.38	1.44
1.4	1.53	1.51
1.6	1.60	1.52
1.8	1.68	1.55

*5 replications, RSD < 4 %

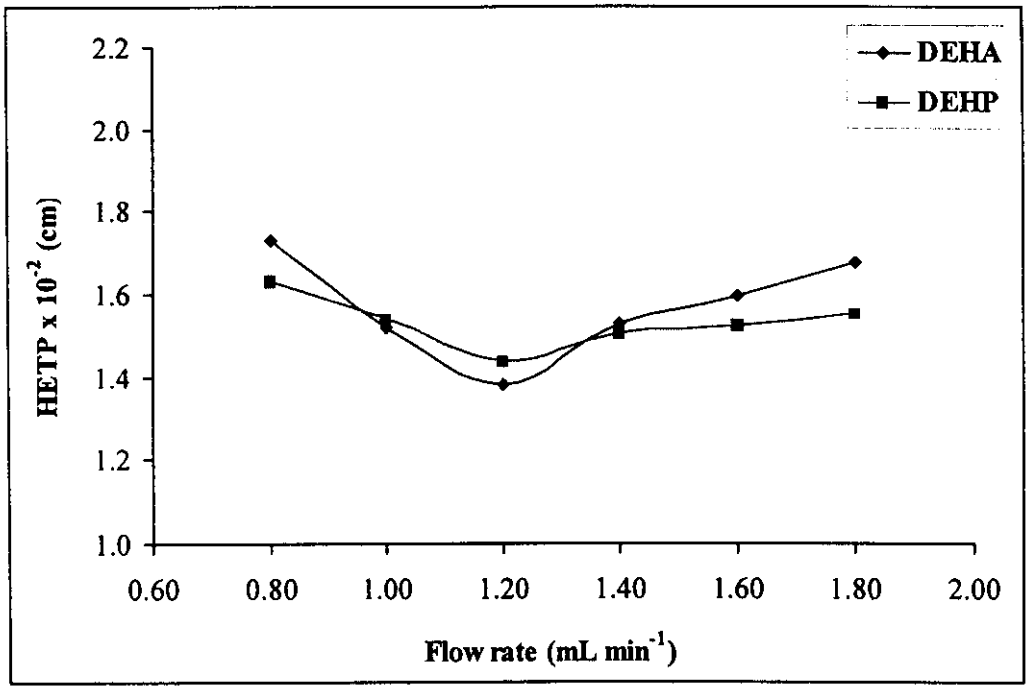

Figure 12 The van Deemter plot of DEHA and DEHP

3.1.2 Column temperature programming

Column temperature is one of the important parameters in gas chromatography technique since it leads to peak resolution. Temperature programming becomes necessary when a sample contains solutes having polarities and/or molecular weights that extend over a wide range. If separated isothermally, it may well result in the less retained solutes being adequately resolved and eluted in a reasonable time. However, the higher molecular weight solutes will be held on the column for an inordinately long period, and the solute peaks, when they are eluted, are likely to be flat which are difficult to evaluate quantitatively (Scott, 1998). The temperature programs were used to minimize the time for eluting/separating the components of interest while narrowing peak widths, increasing integrating detectability and sample throughput and reducing analysis time (Grob, 2004). Therefore, the column temperature programming was applied and optimized (2.5.2). The optimum column temperature programming was obtained by balancing between

the time and response. A temperature program consists of a series of changes in the oven temperature and includes isothermal and controlled temperature rise segments that are selected by a mechanical or microprocessor controller (Poole and Schuette, 1984). In this work, column temperature program consists of initial temperature, hold time of initial temperature, ramp rate of temperature and final temperature.

The results of initial temperature are shown in Table 10 and Figure 13 which indicated that the analysis time decreased when the temperature increased. Initial temperature at 110 °C provided the highest response and a good separation. The response decrease with increasing temperature (after 110 °C) because in gas liquid chromatography (GLC) the separation occur because of selective interactions between the solute and the stationary liquid phase. On the molecular level the principal intermolecular forces that occur between a solute and a solvent are induction and orientation forces that generally weak and decrease with increasing temperature and approach zero at very high temperatures when all orientations are equally probable (Poole and Schuette, 1984). Therefore, the temperature at 110 °C was chosen as the optimum initial temperature.

Table 10 Effect of initial temperature on the response and analysis time of 10 μg mL^{-1},1 μL DEHP-DEHA standard solution

Temperature (°C)	Response (pA*s)*		Analysis time (min)*
	DEHA	DEHP	
90	441±6	529±7	14.50
100	453±3	540±4	14.00
110	461±6	549±5	13.50
120	450±7	539±6	13.00
130	450±5	531±6	12.50
140	445±4	527±6	12.00
150	438±3	520±2	11.50
160	436±3	516±4	11.00

*5 replications, RSD < 4%

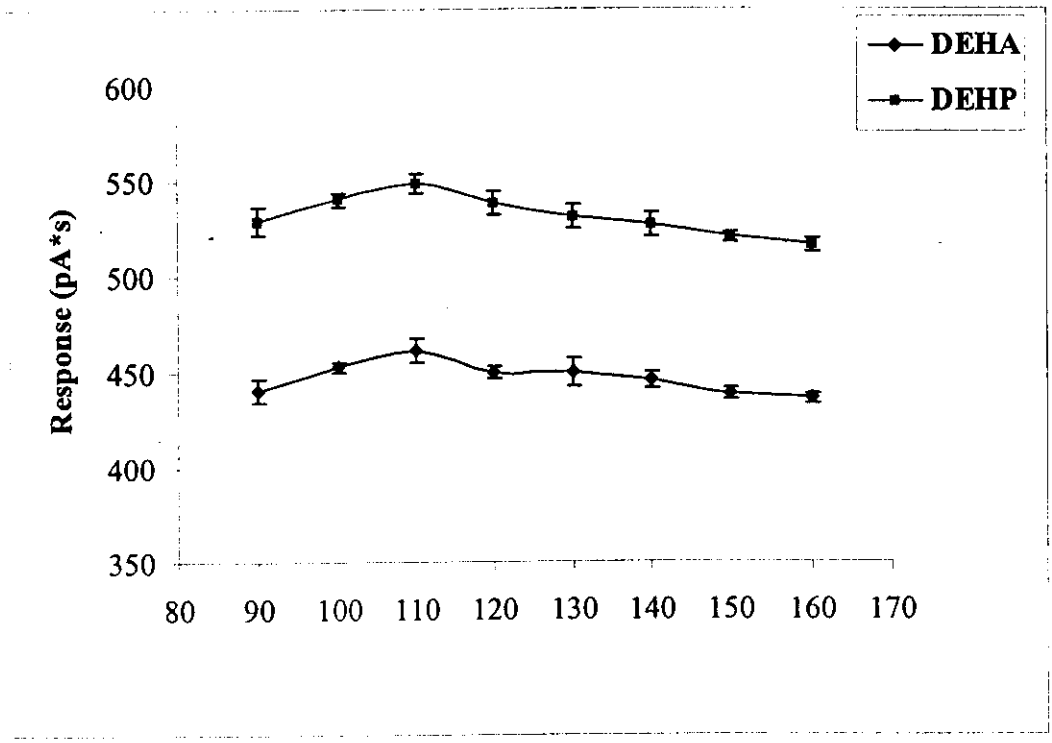

Figure 13 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various initial temperatures

In step *II*, hold time of the initial temperature (110 °C) was investigated and the results are shown in Table 11 and Figure 14. The response increased as the hold time increased from 0 to 1 minute. The response at 1, 2 and 3 minutes differed less than 10 %, therefore, the shorter hold time, 1 minute was chosen as the optimum hold time of the initial temperature.

Table 11 Effect of hold time at the initial temperature on the response and analysis time of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution

Time (min)	Response (pA*s)*		Analysis time (min)*
	DEHA	DEHP	
0	452±5	542±9	13.50
1	513±10	608±9	14.50
2	506±6	608±6	15.50
3	501±4	599±8	16.50

*5 replications, RSD < 4%

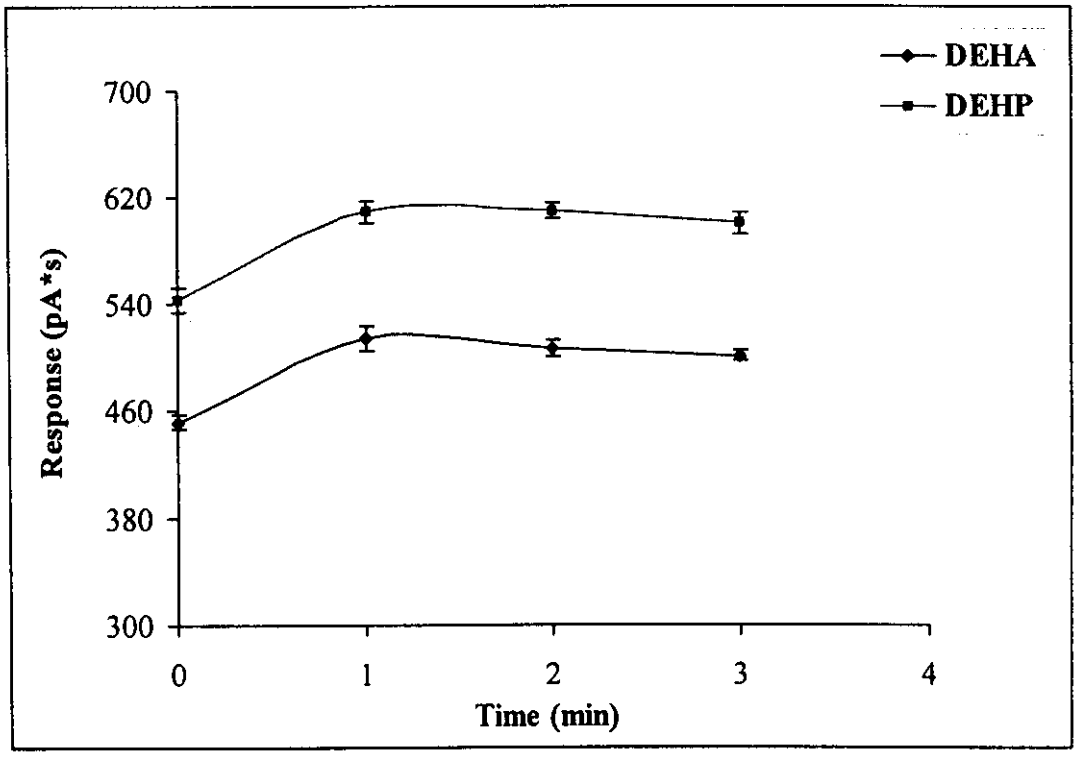

Figure 14 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various holding times

The ramp rate of temperature in step *III* was obtained from experiment in 2.5.2. This was investigated between 10 to 30 °C min^{-1} with an increment of 5 °C min^{-1} as shown in Table 12 and Figure15. The ramp rate of 20 °C min^{-1} provided the highest response. However, ramp rate of 25 and 30 °C min^{-1} gave lower analysis time and the response between 20 and 25 °C min^{-1} differed less than 10% but after ramp rate of 20 °C min^{-1} the baseline drifted. For this reason, the ramp rate of 20 °C min^{-1} was selected as the optimum ramp rate.

Table 12 Effect of ramp rate on the response and analysis time of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution

Ramp rate (°C min^{-1})	Response (pA*s)*		Analysis time (min)*
	DEHA	DEHP	
10	483±3	579±18	24.00
15	494±6	591±8	17.67
20	513±8	609±5	14.50
25	503±7	599±7	12.60
30	496±5	588±6	11.33

*5 replications, RSD < 4%

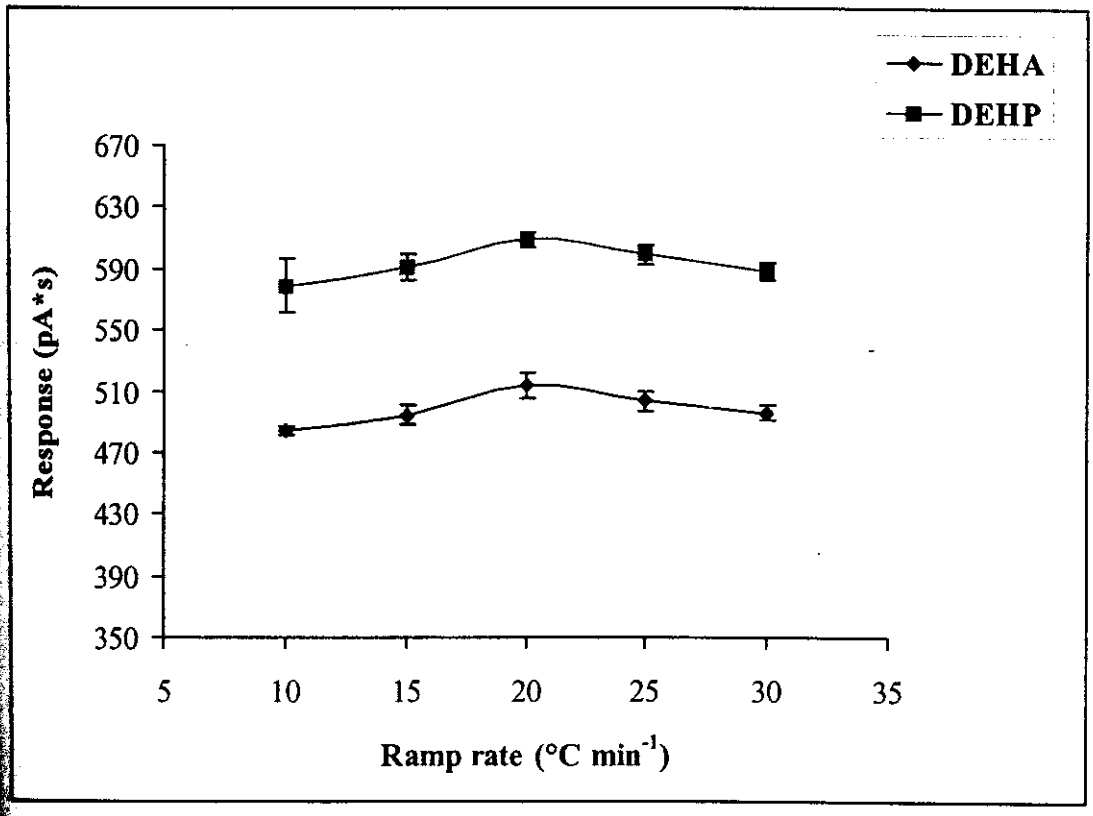

Figure 15 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various ramp rates

The optimum final temperature in step *IV* was investigated by varying the temperature from 260 to 300 °C with an increment of 10 °C. Higher temperature was not tested because the maximum acceptable temperature of HP-5MS column is 325 °C and operating above the recommended temperature will cause column deterioration, and probably detector contamination with consequent increase in noise level and loss of sensitivity (Scott, 1998). The results were obtained as in Table 13 and Figure 16. The response increased as the final temperature increased. The highest response was at 300 °C. Thus, the temperature at 300 °C was selected as the optimum final temperature.

Table 13 Effect of final temperature on the response and analysis time of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution

Temperature	**Response (pA*s)***		**Analysis time**
(°C)	**DEHA**	**DEHP**	**(min)***
260	500±2	596±2	12.50
270	501±10	596±13	13.00
280	507±9	604±7	13.50
290	507±7	603±6	14.00
300	537±7	632±2	14.50

*5 replications, RSD < 4%

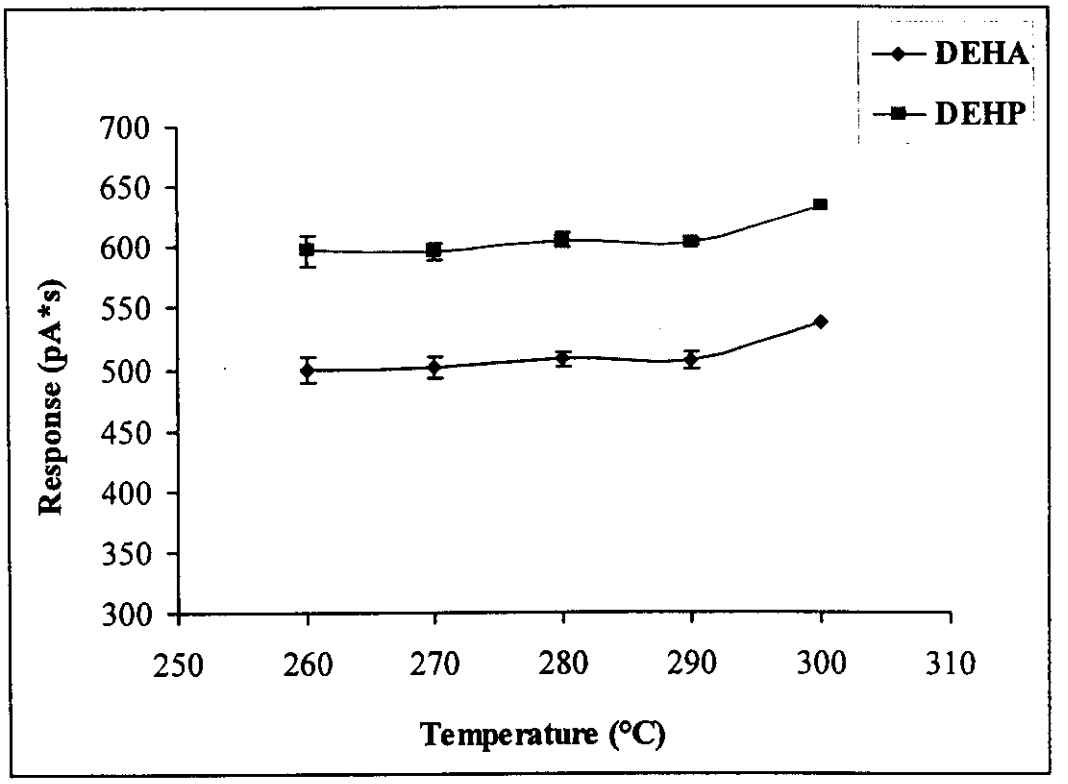

Figure 16 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various final temperatures

After final temperature at 300 °C, the hold time of the final temperature was obtained by considering the time that di (2-ethylhexyl) phthalate (DEHP) could be eluted completely from the column with the optimum conditions. The purpose of holding time of final temperature was for the signal to return to baseline. It had no effect on the response and the baseline resolution. Therefore, to decrease analysis time, the final temperature at 300 °C was only held for 2 minutes. This was enough to allow the signal to go back to the baseline.

In summary, the optimum conditions of the column temperature programming applied in GC-FID was: initial temperature 110 °C, held for 1 minute, ramped at 20 °C min^{-1} to 300 °C and held for 2 minutes (Figure 8). The total analysis time was 14.50 minutes. The results indicated that this could minimize the elution time of DEHP and DEHA with good resolution, increasing detectability and reducing

analysis time. This analysis time was less than the one reported by Chen *et al.*, (2004) and Kim *et al.*, (2003) with the analysis time at 29.00 and 18.00 minutes by GC-MS and GC-FID respectively.

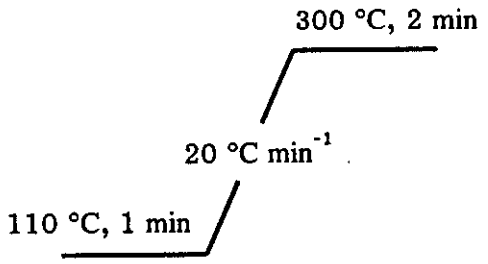

Figure 17 Optimun column temperature programming for DEHP and DEHA analysis

3.1.3 Injector temperature

Injector is an important part of the system that performs the physical task of transferring the sample from a syringe into the gas chromatograph. The injector temperature should be set high enough such that there is enough thermal mass and energy in the inlet to vaporize the injected sample without causing the inlet to cool significantly, but not so high that sample components are decomposed. An inlet is the device on the gas chromatograph that accepts the sample and transfers it to the column. In this work, the splitless inlet was used as shown in Figure 18. It provides a means for improving sensitivity by transferring nearly the entire injected sample into the capillary column rather than venting most of it through the purge vent. In splitless inlet, the purge valve is closed at the moment of injection and remains closed for a period of time (typically 30-60 sec) following the injection. During this period, the sample vapor has no place to go but into the capillary column. When the purge valve is opened, any sample vapor remaining in the inlet is rapidly swept out of the purge valve. Typically, about 95% of the injected sample reaches the capillary column, with sample overload and peak broadening avoided through a series of complex phenomena, related to flow, thermal and solvent effects (Grob, 2004). The principal advantages of the injection using splitless inlet are that rapid vaporization of

the sample is not required (relatively low injection temperatures can be used to minimize sample degradation), it can minimize band broadening by allowing a uniform migration of the solvent (Reedy, 1997), the analysis of very dilute samples is possible without preconcentration, can improve detection limit and the injection device is easily dismantled for removing in volatile sample components (Poole and Schuette, 1985). It is the most commonly used technique for trace (low ppm and ppb analyte concentrations) analyses of compounds.

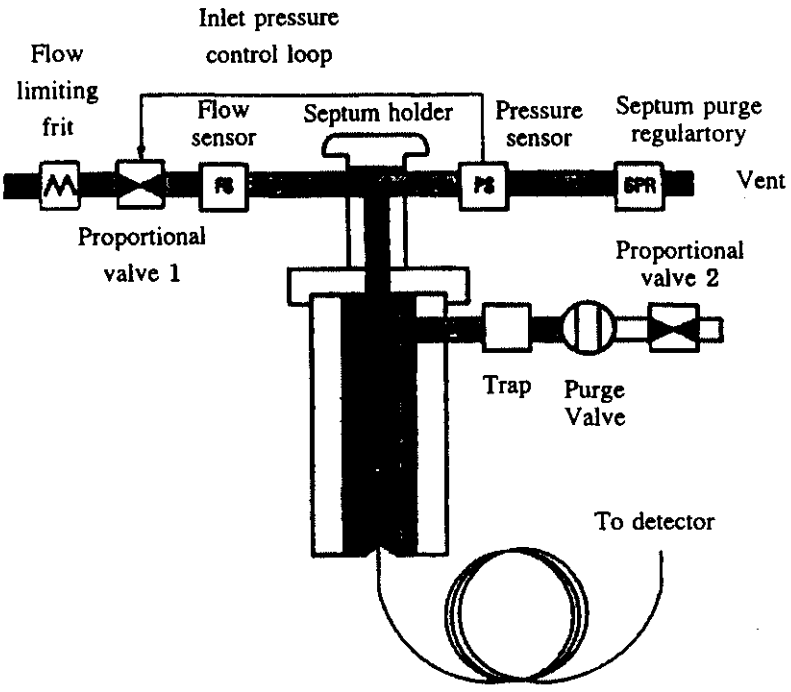

Figure 18 Diagram of spiltless inlet (Agilent Technologies, 1995)

Table 14 and Figure 19 show the response of DEHP and DEHA at injector temperature between 225 to 295 °C. The highest response of these analytes were obtained at 255 °C. After 255°C, the response tends to be constant. The lower injection temperature can minimize the sample degradation and for splitless injection, rapid vaporization of the sample is not required (Poole and Schuette, 1984). Therefore, the temperature of 255 °C was selected to be optimum injector temperature for splitless mode injection analysis.

Table 14 Effect of injector temperature on the response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution

Injector temperature (°C)	Response (pA*s)*	
	DEHA	DEHP
235	484±4	580±5
245	489±17	590±19
255	516±7	614±6
265	493±4	587±6
275	501±9	594±12
285	506±5	598±8
295	501±8	592±9

*5 replications, RSD < 4%

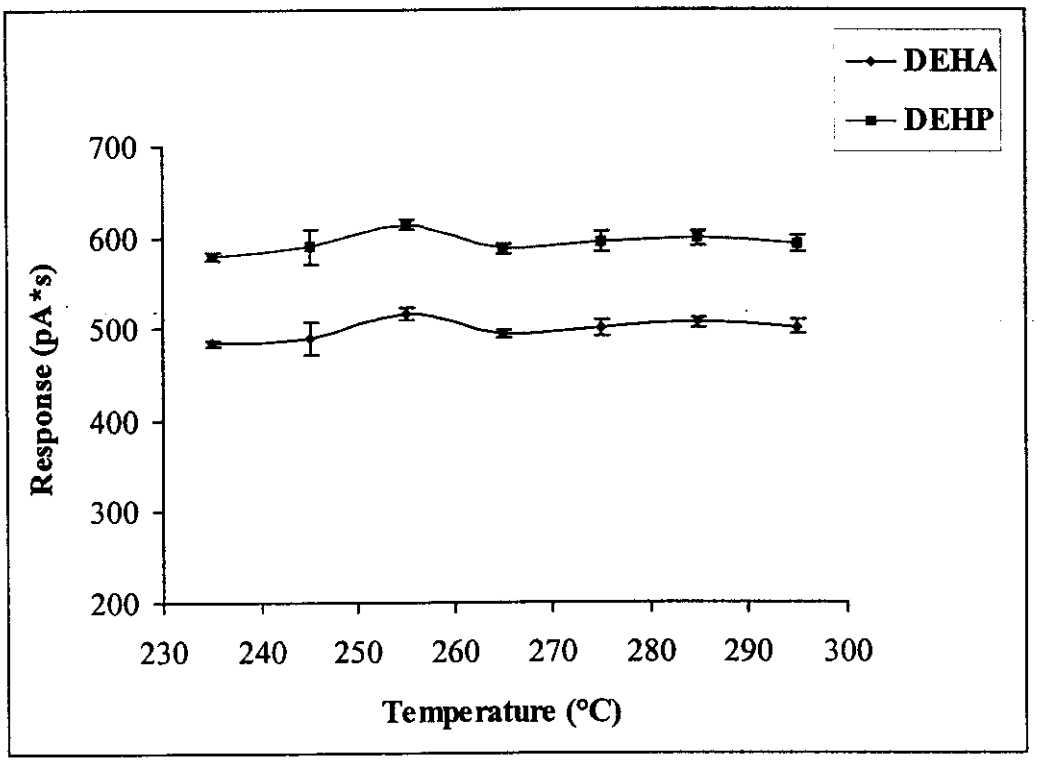

Figure 19 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various injector temperatures

3.1.4 Detector temperature

The detection system in gas chromatography provides the response signal for the chemical compounds separated by the chromatographic column. A flow of finite amounts of chemical entities arrives at the detector in discrete bands, these enties are in the gas phase. The type of detector used depended on the application. In this system the analytes eluted from the column was detected by a flame ionization detector (FID). The FID consists of a small hydrogen-air diffusion flame burning at the end of a jet, to which the eluted components from the column are directed with carrier gas and make up gas flow. The basis of the FID is that the effluent from the column is mixed with hydrogen and burned in air to produce a flame which has sufficient energy to ionize solute molecules having low ionization potentials. The FID is schematically presented in Figure 20 (Grob, 2004), as the organic components reach the flame, electrically charged species are formed, then the charged species are collected at an electrode set at a few volts above the flame. The resulting current is amplified by an electrometer. When carbon-containing compounds are present, ionization occurs and there is a large increase in the electrical conductivity of the flame. Because the sample is destroyed in the flame, a stream-splitting device is employed when future examination of the eluate is necessary: this device is inserted between the column and detector and allows the bulk of the sample to bypass the detector (Mendham *et al.*, 2000).

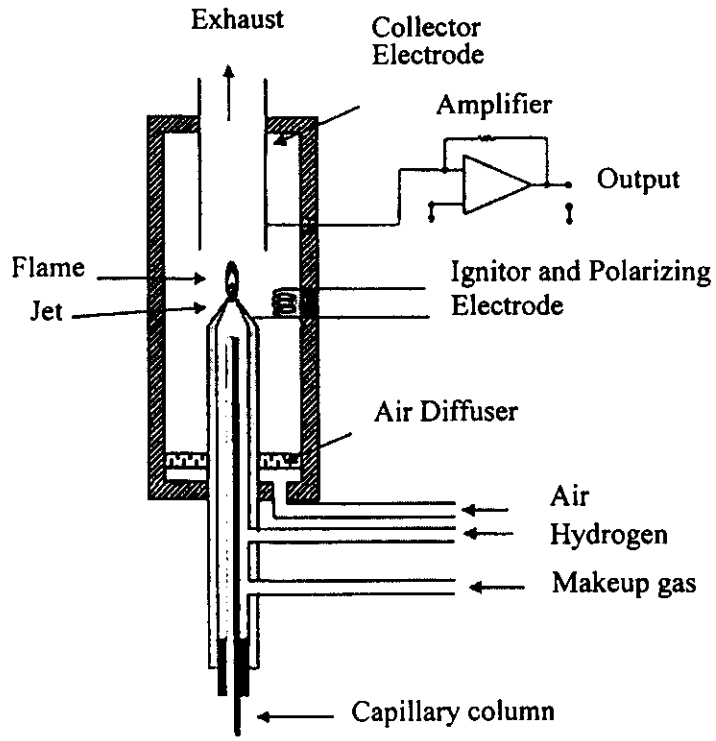

Figure 20 Schematic diagram of a flame ionization detector (FID) (Grob, 2004)

The process in the ionization mechanism in the FID begins at the tip of the jet and occurs in discrete regions of the flame (Figure 21). The mixture of carrier gas, make up gas and hydrogen flows out of the jet and expands outward. Air flows around the outside of the jet. The heat energy produced at the flame reaction zone preheats the flow of gases from the jet by backdiffusion. The organic material eluting from the column undergo degradation reactions in this hydrogen-rich region, forming a group of single carbon species. As the two gas flows mix at the reaction zone, with oxygen available, the following reaction occurs:

$$CH + O^{\bullet} \rightarrow CHO^{+} + e^{-} \qquad (7)$$

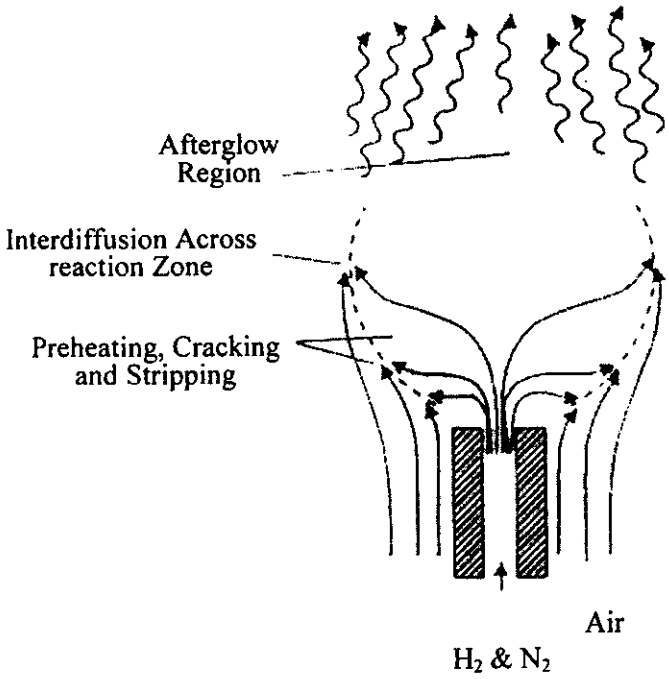

Figure 21 Schematic diagram of flame process in the FID (Grob, 2004)

The $CHO^{\bullet}$ species react rapidly with water produced in the flame to generate hydronium ion:

$$CHO^{\bullet} + H_2O \rightarrow H_3O^{\bullet} + CO \qquad (8)$$

The FID is very nearly a universal detector for gas chromatography of organic conpounds, and coupled with its high sensitivity, stability, fast response and wide linear response range (Mendham *et al.*, 2000). The FID response is the highest for hydrocarbons, being proportional to the number of carbon atoms (Poole and Schuette, 1985), this has made it the suitable detector for analysis of di (2-ethylhexyl) phthalate and di (2-ethylhexyl) adipate that have long chain hydrocarbon.

The optimum detector temperature was selected by considering the effect of the temperature on sensitivity, analysis time and life time of the stationary phase. The detector temperature is always set above 100 °C to prevent

moisture water formation in the combustion process inside the detector (Grob, 2004). The responses from various detector temperatures are shown in Table 15 and Figure 22. The relationship between detector temperature and the response indicated that the response increased with temperatures. The highest response for DEHP and DEHA was obtained at 300 °C. Higher temperature was not tested because in FID, the tip of capillary column is inserted into the end of an FID jet (see Figure 20). This approach has been used to improve peak shape since it prevents the loss of the integrity due to adsorption or possible catalytic decomposition of compounds as they come in contact with hot metal surfaces (Grob, 2004). From this reason, the detector temperature will affect the column. According to the maximum acceptable temperature of HP-5MS column is 325 °C. If operating above the recommended temperature it will cause column deterioration (Scott, 1998). Therefore, the temperature at 300 °C was selected to be the optimum detector temperature.

Table 15 Effect of detector temperature on the response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution

Detector temperature (°C)	**Response (pA*s)***	
	DEHA	**DEHP**
260	472±11	569±15
270	487±4	580±4
280	498±9	591±9
290	499±11	593±12
300	517±7	615±7

*5 replications, RSD < 4%

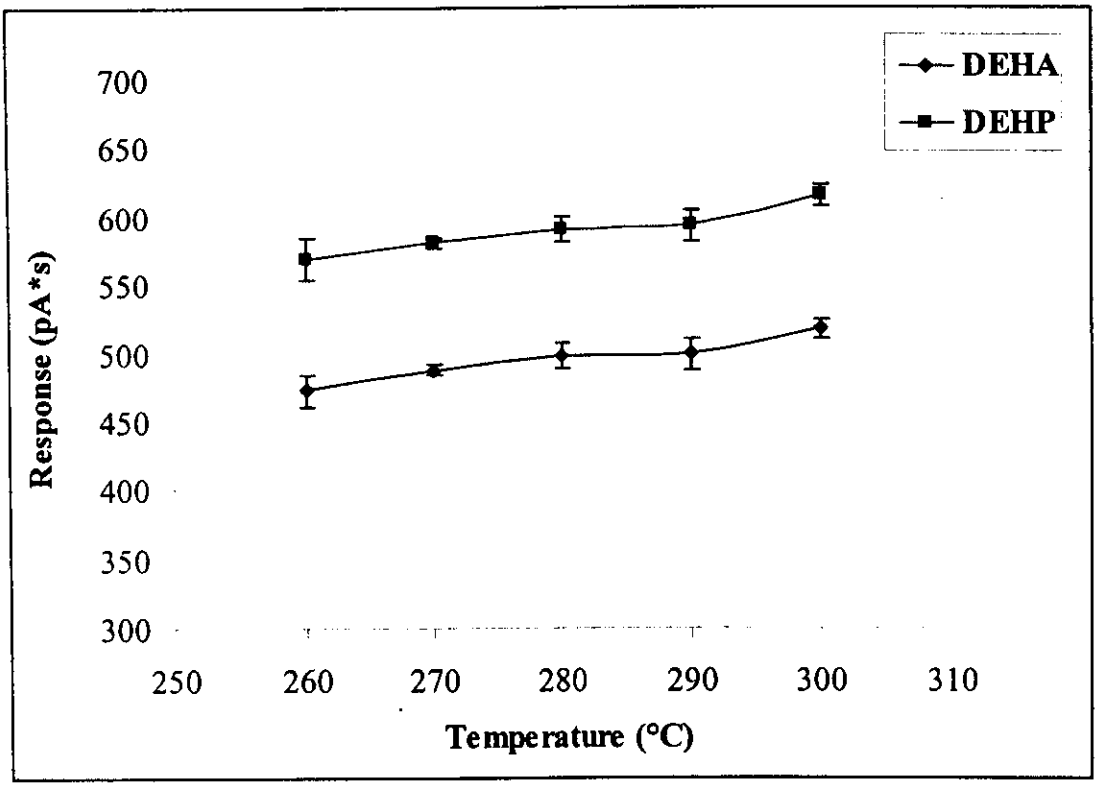

Figure 22 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various detector temperatures

3.1.5 Fuel (H_2) flow rate

The FID detector operated in a hydrogen-rich mode and using oxidant (air) to support combustion. Therefore, it required fuel (hydrogen gas, H_2) and oxidant to make the flame and flow rates of hydrogen and air can significantly influence the detector sensitivity and the noise level. The effect of fuel gas flow rate on the responses are shown in Table 16 and Figure 23. The responses were approximately the same from 30 to 50 mL min^{-1}. The highest response was obtained at 40 mL min^{-1}. However, when consider the responses at 30 and 40 mL min^{-1}, they differed by less than 10%. Therefore, to reduce cost, the flow rate at 30 mL min^{-1} was selected as the optimum flow rate and this is similar to the recommendation by GC-FID manual (Agilent, 1995).

Table 16 Effect of fuel gas flow rate on the responses of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution

Hydrogen flow rate ($mL\ min^{-1}$)	Response (pA*s)*	
	DEHA	DEHP
20	384±9	459±9
30	507±7	606±7
40	508±19	608±19
50	462±14	555±15

*5 replications, RSD < 4%

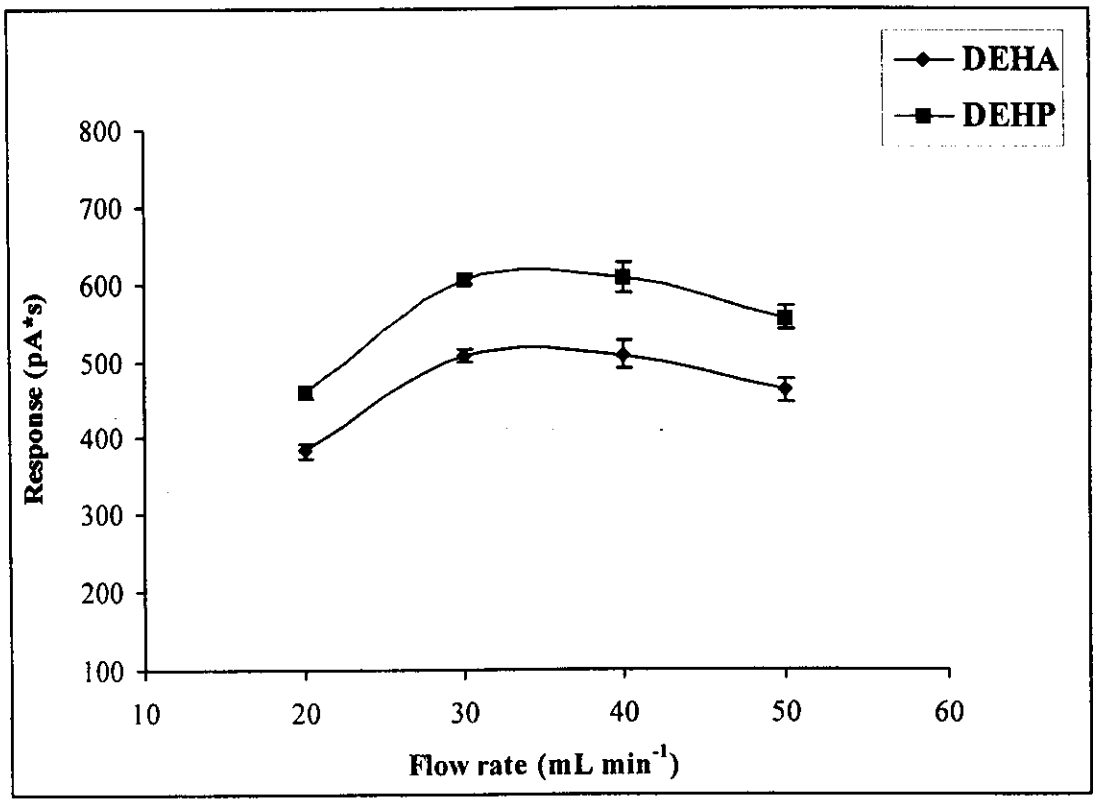

Figure 23 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various fuel gas flow rates

3.1.6 Oxidant (air) flow rate

In this study, air was used as an oxidant for FID. Typical flows for air are 300-500 mL min^{-1} (Grob, 2004). The effect of air flow rate on the responses is shown in Table 17 and Figure 24. The flow rate of 300 mL min^{-1} gave the highest response and the data is the most precise due to the lowest standard deviation (SD). Thus this flow rate was chosen as the optimum condition. Therefore, the optimum fuel and air flow rate was 1:10. This ratio agreed well with most GC-FID systems (Poole and Schuette, 1984).

Table 17 Effect of oxidant gas (air) flow rate on the responses of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution

Air flow rate (mL min^{-1})	**Response (pA*s)***	
	DEHA	**DEHP**
100	456±9	547±12
200	471±13	565±17
300	497±2	592±2
400	485±13	578±15

*5 replications, RSD < 4%

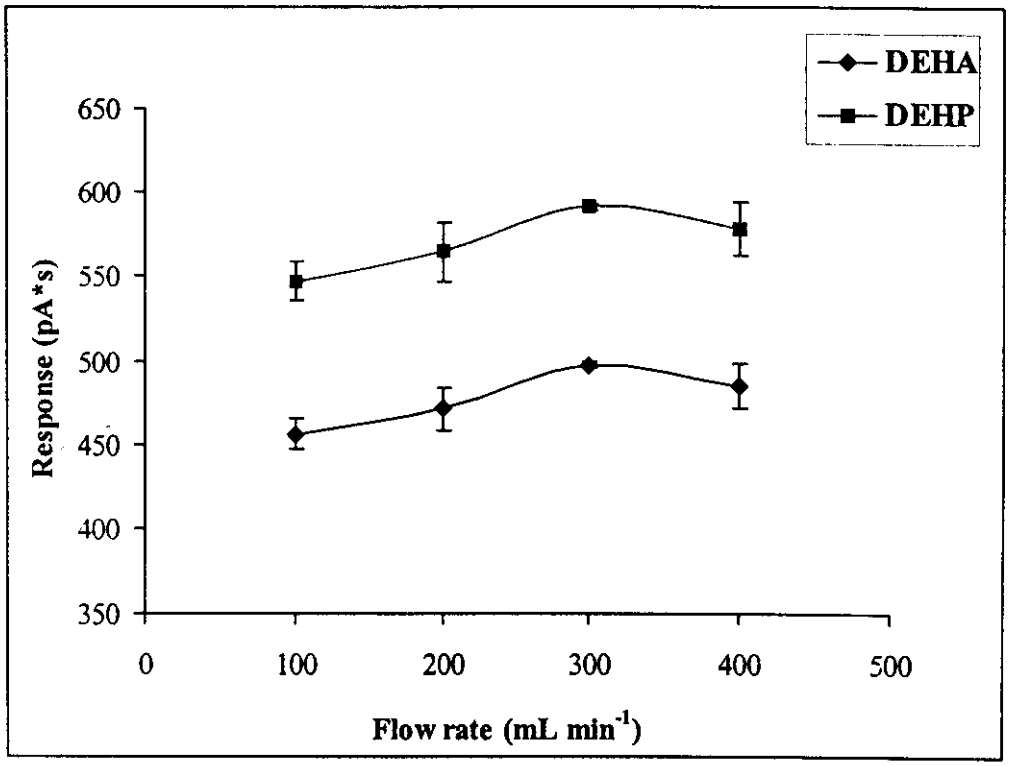

Figure 24 Response of 10 μg mL^{-1}, 1 μL DEHP-DEHA standard solution at various oxidant gas flow rates

A makeup gas was used to carry the analyte in the column to the detector zone. It is added to the stream just before the analyte enter to the detector. The FID response is greater with nitrogen as a makeup gas (Grob, 2004). In this study, the flow rate of nitrogen was applied at 30 mL min^{-1} as recommended by the manufacturer that it should be in the range of 10 to 60 mL min^{-1}, with a recommended value of 30 mL min^{-1}.

3.1.7 Summary of GC-FID conditions

The optimum conditions for di (2-ethylhexyl) phthalate (DEHP) and di (2-ethylhexyl) adipate (DEHA) analysis on capillary column (HP-5MS, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) with flame ionization detector are summarized in Table 18. The chromatograms obtained using these optimum conditions is shown in Figure 25.

Table 18 Optimum conditions of GC-FID for phthalate and adipate esters analysis

Conditions	Optimum values
Flow rate: He, carrier gas	1.2 mL min^{-1}
H_2, fluel gas	30 mL min^{-1}
Air, oxidant gas	300 mL min^{-1}
Column temperature program:	
Initial temperature	110 °C
Initial hold time	1 minute
Ramp rate	20 ° C min^{-1}
Final temperature	300 ° C
Final hold time	2 minutes
Injector temperature	255 °C
Detector temperature	300 °C

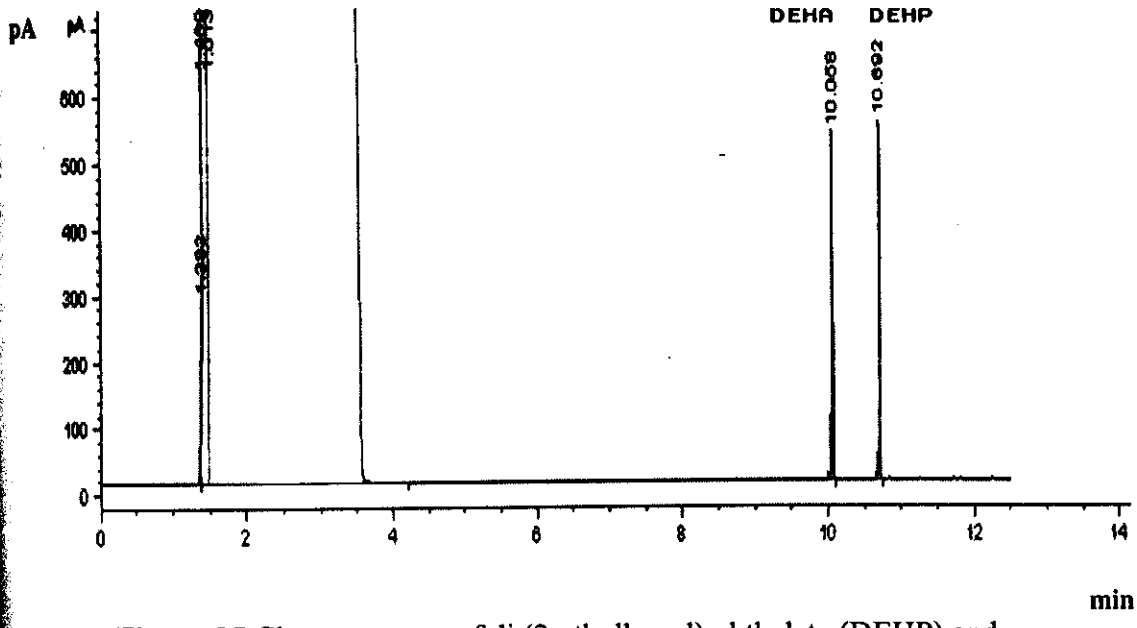

Figure 25 Chromatogram of di (2-ethylhexyl) phthalate (DEHP) and di (2-ethylhexyl) adipate (DEHA) at optimum GC-FID conditions

3.2 Limit of detection (LOD)

The limit of detection (LOD) is the lowest concentration or amount of analyte that the detector could confidently detect by the method (Eurachem, 1998). Several approaches for determining the detection limit are possible, depended on the procedure either non-instrumental or instrumental. In this work, the detection limit was based on the signal to noise ratio. This ratio is indicative of the probability that a particular peak; in a noisy baseline, represents the signal from analytes (Grob, 2004). The signal to noise ratio can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal to noise ratio is determined by serial dilution of standard solution (Mitra, 2003) and comparing measured signals from known concentrations of analytes with noise and establishing the minimum concentration at which the analytes can be reliably detected. A signal to noise ratio 3:1 is generally considered acceptable for estimating the detection limit (ICH-Q2B, 1996).

In this work, the limits of detection for phthalate and adipate esters are shown in Table 19. The different efficiency of the FID to each analytes was due to their different structures (see in chapter1). The LOD for DEHP and DEHA were 25 and 12 ng mL^{-1} respectively.

Table 19 Limit of detection of DEHP and DEHA with S/N > 3

Analytes	**Limit of detection (ng mL^{-1})**
DEHP	25
DEHA	12

*5 replications, RSD < 4%

Table 20 LOD reported for the determination of DEHP (Gómez-Hens and Aguilar-Caballos, 2003)

Sample	Method[a]	LOD (ng g^{-1})	Reference
Toys	SE-GC-FID	5000	Rastogi, 1998
Foods	SE-GC-MS	10	Gruber *et al.*,1998
Foods	SE-GC-MS	23	Tsumaru *et al.*, 2001
Foods	SE-SPE-GC-MS	70	Petersen *et al.*, 2000
Baby foods	SE-SPE-GC-MS	37	Tsumaru *et al.*, 2002

[a]SE: solvent extraction, SPE: Solid phase extraction

The reported limit of detections for the determination of DEHP with other methods were as shown in Table 20. The LOD of DEHP in this work (25 ng mL^{-1}) is nearly the same as Tsumura *et al.* (2001), using mass spectrometry detector. When comparing the LOD of this method with those reported by Rastogi (1998), Petersen *et al.* (2000) and Tsumura *et al.* (2002), it can be seen that this method give lower LOD.

3.3 Linear dynamic range (Linearity)

Linear dynamic range or linearity of an analytical method is the ability to obtain test results of variable data which are directly propotional to the concentration (amount of analyte) in the sample. The data variable used for quantitation of the analytes is peak area (Chaung Chow, 2004). In experiment 2.7, the dynamic range was investigated by serial dilutions of a stock standard solution. Linear dynamic range can be obtained from a calibration curve, which is the relationship between instrument response and known concentration of the analytes. Linearity is achived when the coefficient of determination (R^2) is equal or greater than 0.99 (FDA, 2000 and Chung Chow, 2004). The slope of the regression line will provide the

sensitivity of the regression and hence the method to be validated. The *y*-intercept will provide the analyst with an estimate of the variability of the method (Chung Chow, 2004). Table 21 and Figure 26 show the response of DEHA and DEHP at various concentrations. The system provided a wide linear dynamic range from 25 ng mL^{-1} to 60 μg mL^{-1} with $R^2 > 0.99$ and the relative standard deviations (RSD) lower than 4%.

Table 21 Response of DEHA and DEHP at various concentrations

Concentration (μg mL^{-1})	**Response (pA*s) × 10^2***	
	DEHA	**DEHP**
0.025	0.0280±0.0009	0.028±0.002
0.05	0.030±0.002	0.380±0.003
0.10	0.070±0.004	0.16±0.01
0.50	0.330±0.009	0.380±0.006
0.75	0.500±0.001	0.57±0.02
1	0.50±0.01	0.600±0.009
5	2.60±0.03	3.00±0.03
10	50.00±0.02	58.00±0.02
20	10.0±0.1	12.0±0.1
40	19.0±0.2	22.0±0.3
60	31.0±0.5	36.0±0.5

*5 replications, RSD < 4%

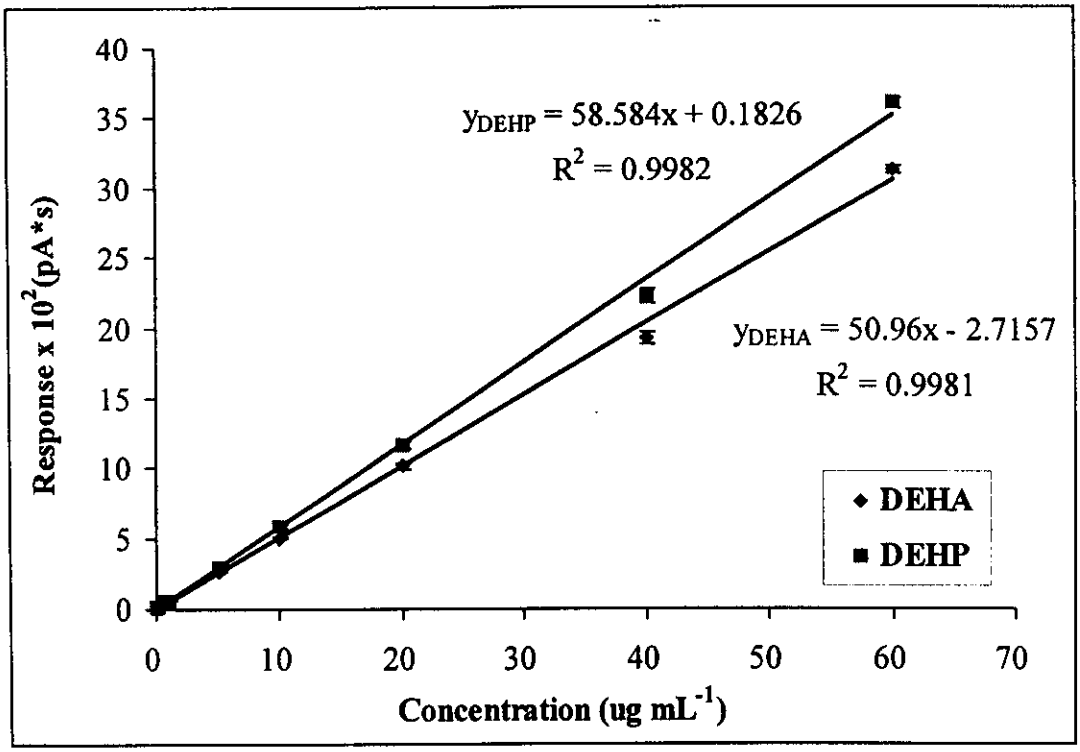

Figure 26 Linear dynamic range of DEHP and DEHA

3.4 Sample preparation

Application of analytical method is always easier when the matrix does not contain species that interfere with determination of the analyte. However, when an interference is expected, it is necessary to isolate the component to be measured from the matrix. Therefore, the quality of the result often depends on sample preparation. This preliminary step can have an important influence on the end result (Rouessac and Rouessac, 2000). In this work, the analysis of phthalate and adipate esters in packaged food is based on ultrasonic extraction followed by solid phase extraction (SPE) for sample preparation and analyzed by gas chromatography coupled with flame ionization detector (GC-FID).

3.4.1 Optimization of ultrasonic extraction

Ultrasonic extraction uses mechanical energy in the form of a shearing action, which is produced by a low-frequency sound wave. The sample is immersed in an ultrasonic bath with solvent and subjected to ultrasonic wave (LeBlanc, 2001). Then, the sample is separated from the extract by vacuum filtration as described in 2.8.3.

3.4.1.1 Extraction time

Ultrasonic extraction or sonication technique, can be used to get faster and more complete extraction. The ultrasonic agitation allows more intimate solid-liquid contact and the gentle heating that results during sonication can aid the extraction process. Sonication is also a recommended procedure for the pretreatment of solid environmental samples (Settle, 1997). The influence of the extraction time was investigated by monitoring the variation of analytical signal as a function of exposure time (Table 22 and Figure 27). From the results, the extraction efficiency increases with time but at extraction times longer than 90 minutes, a decrease in the responses of both DEHA and DEHP were observed. This may be explained considering the possibility of organic compounds (extraction solvent) decomposition by the effect of the sound waves. This is one of the limitations of this technique (Melecchi *et al.*, 2003). Therefore, the extraction can be considered to be completed in 90 minutes and this was selected (this time also gave the lowest standard deviation (SD)).

Table 22 Effect of extraction time on the response of DEHA and DEHP extraction

Concentration ($\mu g\ mL^{-1}$)	Response (pA*s)*	
	DEHA	DEHP
30	158±6	160±11
60	165±4	169±7
90	182±0	187±2
120	175±2	178±5
150	144±1	176±5

*5 replications, RSD ≤ 7%

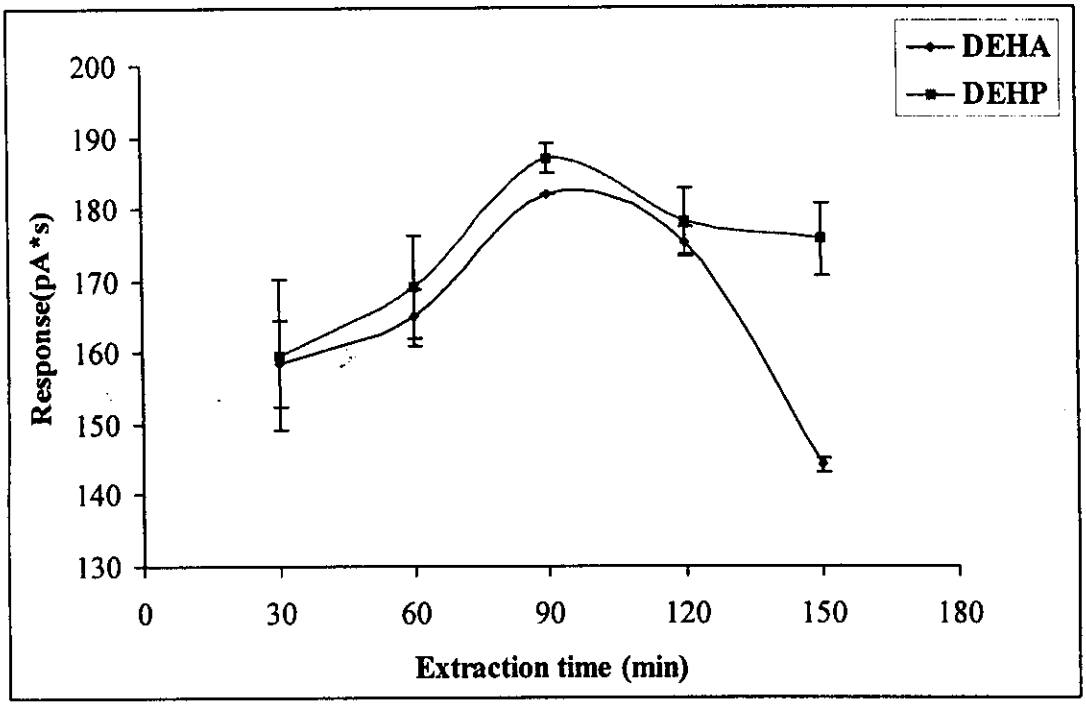

Figure 27 Responses of DEHP and DEHA at various extraction times

3.4.1.2 Extraction solvent

The selection of an appropriate extraction solvent is important for the optimization of the ultrasonic extraction process. The purpose of this experiment was to assess the extraction ability of several solvents by the sonication method. Five solvents with different polarity and water solubility were used for extracting DEHP and DEHA and the effect is shown in Table 23 and Figure 28. The most appropriate solvent for extracting DEHP and DEHA was the mixture of dichloromethane and cyclohexane 1:1 (v/v) which gave the highest response.

Extraction efficiency of any solvent depends on polarity index, dipole moment, dielectric constant and solubility. Polarity is the sum of all molecular properties responsible for all the interaction forces between solvent and solute molecules that lead to the overall salvation ability of the solvent (Marcus, 1998). Normally, the organic solvents that have high polarity will have high dielectric constant and in contrast, solvents that have low polarity will have low dielectric constant. In addition, solvents with high dipole moment will have a strong polarity. The dipole moment of the solvents are therefore, corresponded to their polarity index. Table 24 shows polarity index, dipole moment and dielectric constant of acetonitrile, cyclohexane, ethyl acetate, dichloromethane, hexane and acetone.

According to the structure of DEHP and DEHA and their physical properties, DEHP is non-polar and DEHA is moderately polar due to its weak solubility in water at 0.78 mg L^{-1} at 22 °C (Environmental Health Hazard Assessment, OEHHA, 2002). Therefore, solvents with similar properties would provide good extraction efficiency. That is why the mixture of dichloromethane (slightly polar) and cyclohexane (non polar) provide the best response for the extraction of DEHP and DEHA. In the case of hexane, although it has similar polarity as cyclohexane but hexane vaporizes more easily than cyclohexane (because its volatility is higher, the volatility of hexane and cyclohexane are 2.02×10^{1} and 1.30×10^{1} kPa (Marcus, 1998), repectively). This may cause more loss of hexane than cyclohexane during the extraction process. Since DEHP and DEHA could partition into the mixture of dichloromethane and cyclohexane 1:1 (v/v) better than other solvents, it was selected to be the solvent for the extraction of phthalate and adipate

esters. This result was similar to the one obtained by Lau and Wong (1996) where they used cyclohexane-dichloromethane (1:1) (v/v) as an extraction solvent for extracting plasticisers from fatty and non-fatty food with 90 to 106% recoveries.

Table 23 Effect of extraction solvent on the response of DEHA and DEHP

Type of solvent	Response (pA*s)*	
	DEHA	DEHP
Hexane-acetone 1:1 (v/v)	136±2	147±4
Hexane-dichloromethane 1:1 (v/v)	114±3	117±5
Dichloromethane-cyclohexane 1:1 (v/v)	158±3	178±7
Acetonitrile	139±4	138±8
Ethyl acetate	155±6	142±7

*5 replications, RSD ≤ 6%

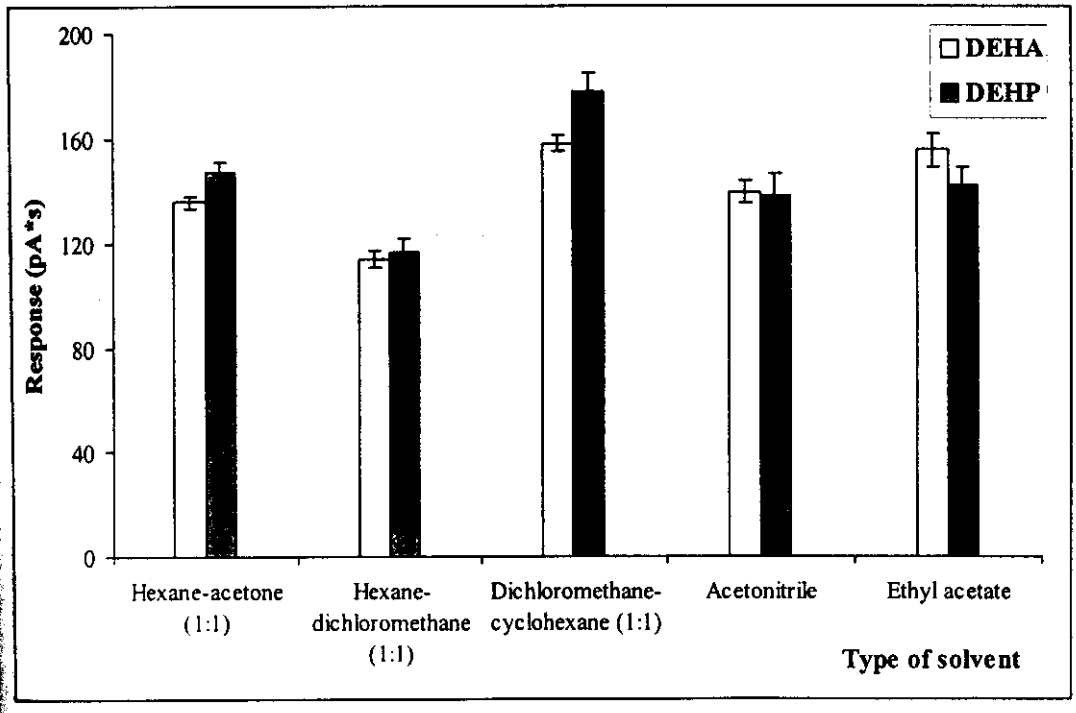

Figure 28 Responses of DEHP and DEHA at various extraction solvents

Table 24 Polarity index, dipole moment and dielectric constant of solvents

Solvents	Polarity index*	Dipole moment**	Dielectric constant***
Cyclohexane	0.04	0.0	2.0
Hexane	0.1	0.1	2.0
Dichloromethane	3.4	1.6	9.1
Ethyl acetate	4.4	1.8	6.0
Acetone	5.4	2.9	20.7
Acetonitrile	5.8	3.9	37.5

*Skoog *et al.*, 1998; Waters, 1997

***Dielectric constant reference guide, 1998

3.4.1.3 Volume of extraction solvent

The effect of solvent volume on the extractability of DEHP and DEHA from curry paste sample was evaluated. The results (Table 25 and Figure 29) showed that the responses of 20 and 30 mL were higher than 10 and 40 mL. Extraction volume of 20 mL was chosen because the responses at 20 and 30 mL differed less than 10% and the less solvent used can reduce the hazardous waste.

Table 25 Effect of extraction volume on the response of DEHA and DEHP extraction

Volume (mL)	Response (pA*s)*	
	DEHA	DEHP
10	114±9	100±11
20	167±10	180±5
30	174±9	179±13
40	148±10	142±12

*5 replications, RSD ≤ 11%

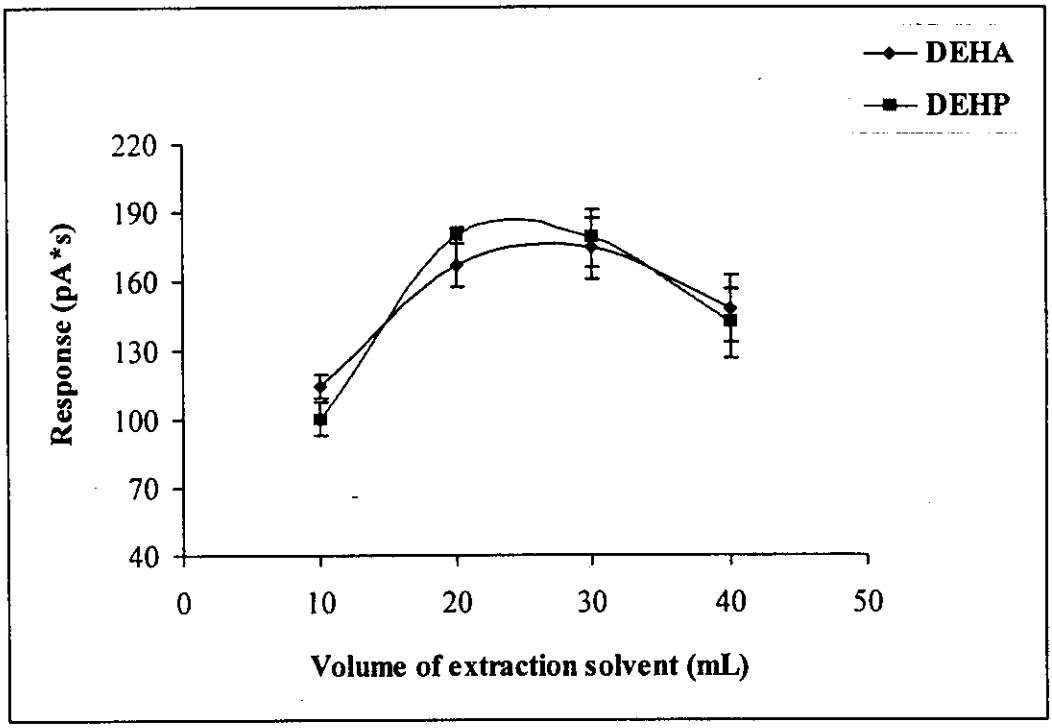

Figure 29 Responses of DEHP and DEHA at various volumes of extraction solvent

3.4.2 Optimization of solid phase extraction

After ultrasonic extraction, the extracts were then cleanup by SPE. The purpose of applying a cleanup method to an extract is to reduce the

interferences such as lipids, compounds of the matrix, which could interfere and cause poor analytical results (Sablayrolles, 2005; Jiménez, 2001 and EPA method 3600C) with the later's final determination and quantification with a minimal loss of the analyte (Ferrer *et al.*, 2005).

There are two basic methods for sample cleanup: the sample solution is passed through the cartridge with the object of either retaining the analytes of interest while the matrix components pass through or retaining the matrix components while the analytes pass through. (Fifield and Kealey, 2000; ATSDR, 2002; Altech, 2002), the latter method was used in this work. The sample extracts are purified by means of solid phase extraction with Florisil® as the stationary phase. Using Florisil® cartridges, analytes can be quantitatively extracted from the sample with an appropriate organic solvent; while fat, and other potential interferences, were retained on the sorbent material. Relatively clean extracts were obtained (Criado *et al.*, 2004).

3.4.2.1 Sample flow rate

One of the most neglected considerations in the practice of SPE is the control and optimization of flow rate. The flow rate of the sample solution through the sorbent is important. This is because mass transfer resistance affects the chromatographic performance of the cartridge (Meloan, 1999). It not only affects the responses of the analytes, but also control the time of analysis (Cai *et al.*, 2003). The flow rate is independent of retention capacity, and much more to do with the kinetic sorptive properties of SPE. In other words, when the flow rate is too high for a given SPE step, it will allow minimal time for analyte-sorbent interaction so analyte molecules pass through the SPE cartridge without sufficient residence time to properly adsorb or desorb from the SPE sorbent. This must be balanced against the need to pass the entire sample through the cartridge. For sample load, premature breakthrough can occur (Sigma-Aldrich, 2005). In this case, loading sample is chosen to let the analytes of interest pass through the cartridge, while the interferences are adsorbed on the sorbent. Normally a flow rate of 3-10 ml min^{-1} was use to operate for SPE cartridge (Dean, 1998). Table 26 and Figure 30 show the responses of DEHP and

DEHA at different sample flow rates. The flow rate of 3 mL min^{-1} provided the highest response and it was selected as the optimum sample flow rate.

Table 26 Effect of sample flow rate on the response of DEHA and DEHP extraction

Flow rate (mL min^{-1})	Response (pA*s)*	
	DEHA	DEHP
1	170±9	172±11
3	177±10	180±5
5	172±9	171±13
7	167±10	164±12

*5 replications, RSD ≤ 8%

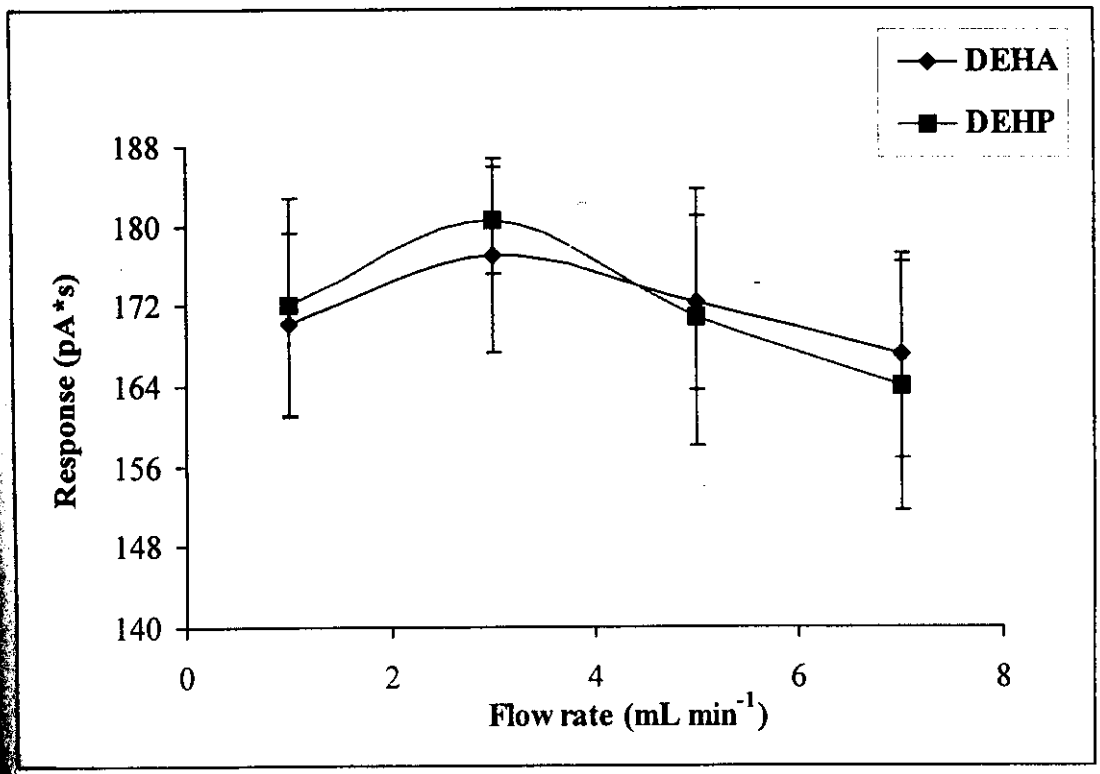

Figure 30 Responses of DEHP and DEHA at various sample flow rate

3.4.2.2 Drying time

Because the elution solvent is water-immisible, it was important to remove the residual water from the SPE cartridge prior to the elution of DEHP and DEHA. Therefore, drying time of SPE cartridge after sample loading is also an important aspect to be taken into account. Table 27 and Figure 31 show the response at different drying times where the drying time of 3 minutes gave the highest response and was selected as the optimum time to dry the cartridge after sample loading.

Table 27 Effect of drying time on the response of DEHA and DEHP

Drying time (min)	Response (pA*s)*	
	DEHA	DEHP
0	169±14	167±13
3	170±14	171±16
6	186±10	193±13
9	154±14	151±14

*5 replications, RSD ≤ 10%

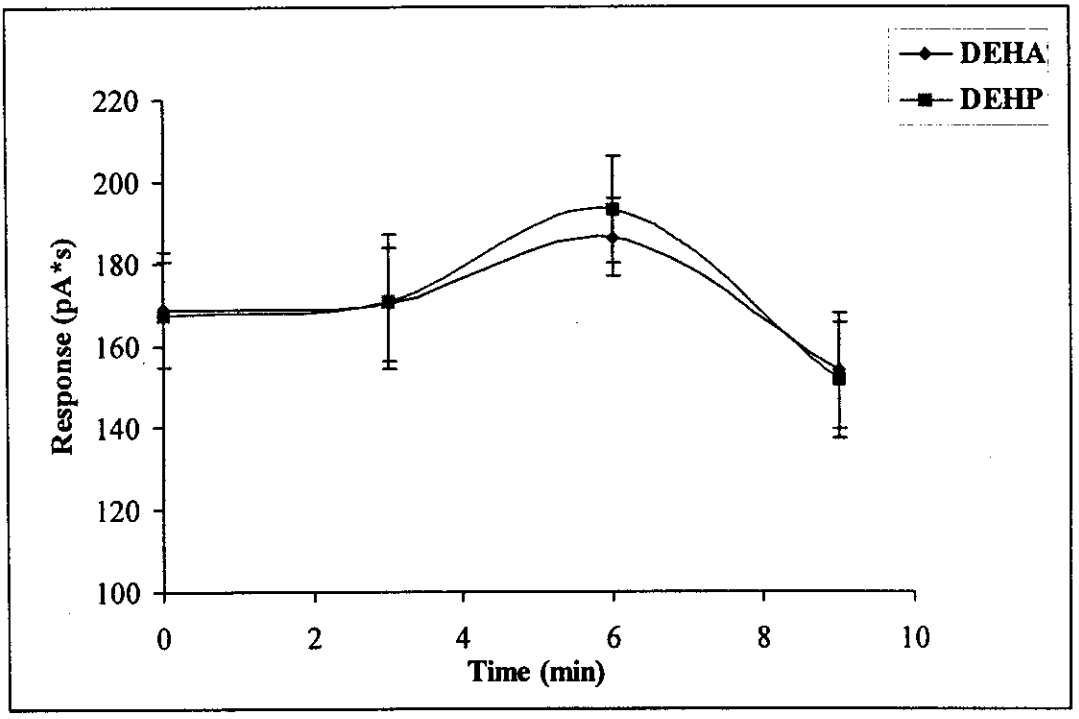

Figure 31 Responses of DEHP and DEHA at various drying times

3.4.2.3 Type of eluting solvent

The choice of solvent directly influences the retention of the analytes on the sorbent and its subsequent elution, whereas the solvent polarity determines the solvent strength (or ability to elute the analytes from the sorbent in a smaller volume than a weaker solvent) (Dean, 1998). To investigate the elution characteristics of the SPE cartridge, a 1 mL aliquot of the extractant was loaded on to the Florisil® cartridge and the analytes were then eluted with various eluting solvents as decribed in 2.8.3.4. The SPE step in this work use a procedure by which compounds of interest are not retained on the packing, thus, removal of any residual, desired components from the cartridge was necessary to complete the extraction process (Supelco, 1998). The elution profiles of DEHP and DEHA are shown in Table 28 and Figure 32.

Acetone-hexane (10/90, v/v) provided the best extraction efficiency while ethyl acetate and 50/50 (v/v) hexane-diethyl ether also gave nearly

the same response to acetone-hexane (10/90, v/v). This could be explained as follows. Since DEHP is a non-polar compound and DEHA is more polar than DEHP, some molecules of DEHA will be retained on Florisil® adsorbent surface with hydrogen bonding while DEHP will pass through the sorbent into the collection test tubes. In the elution step, DEHP and DEHA residuals are eluted by passing a solvent that disrupts the binding mechanism. The polarity of elution solvent should be strong enough to completely elute DEHP and DEHA residuals. The addition of acetone help to increase the polarity of eluting solvent. For ethyl acetate, when compared with acetone-hexane (10/90, v/v), it gave lower response and the response of DEHP differed more than 10%. In addition, ethyl acetate is a general solvent in coatings and plastics, organic synthesis (Lewis, 1993). Therefore, it may be contaminated during SPE process such as in eluting step if ethyl acetate is used as an eluting solvent. The source of contamination may also come from Teflon (polytetrafluoro ethylene, PTFE) tube that used in SPE manifold because from the report by Agency for Toxic Substances and Disease Registry (ATSDR), the presence of DEHP as a contaminant in Teflon has been found (ATSDR, 2002). For 50/50 (v/v) hexane-diethyl ether, it is not suitable to be used as solvent because it is highly volatility and flammable. Diethyl ether is also more toxic than other solvents. High concentration of diethyl ether vapors may induce unconsciousness, dizziness, vomiting, weakness, headache and cyanosis (Lab-Scan Analytical Science, 2003). Therefore, the appropriate eluting solvent was acetone-hexane (10/90, v/v). This result was similar to Sablayrolles *et al.* (2005) and EPA Method 3620B (1996).

Table 28 Effect of eluting solvent on the response of DEHA and DEHP

Type of eluting solvent	Response (pA*s)*	
	DEHA	DEHP
Ethyl acetate	175±3	190±9
Hexane-diethylether (50/50, v/v)	173±14	199±18
Acetone-hexane (10/90, v/v)	188±8	216±8
Dichloromethane- cyclohexane (1:1, v/v)	176±16	181±14
Dichloromethane-hexane (4:1, v/v)	162±11	171±18

*5 replications, RSD ≤ 10%

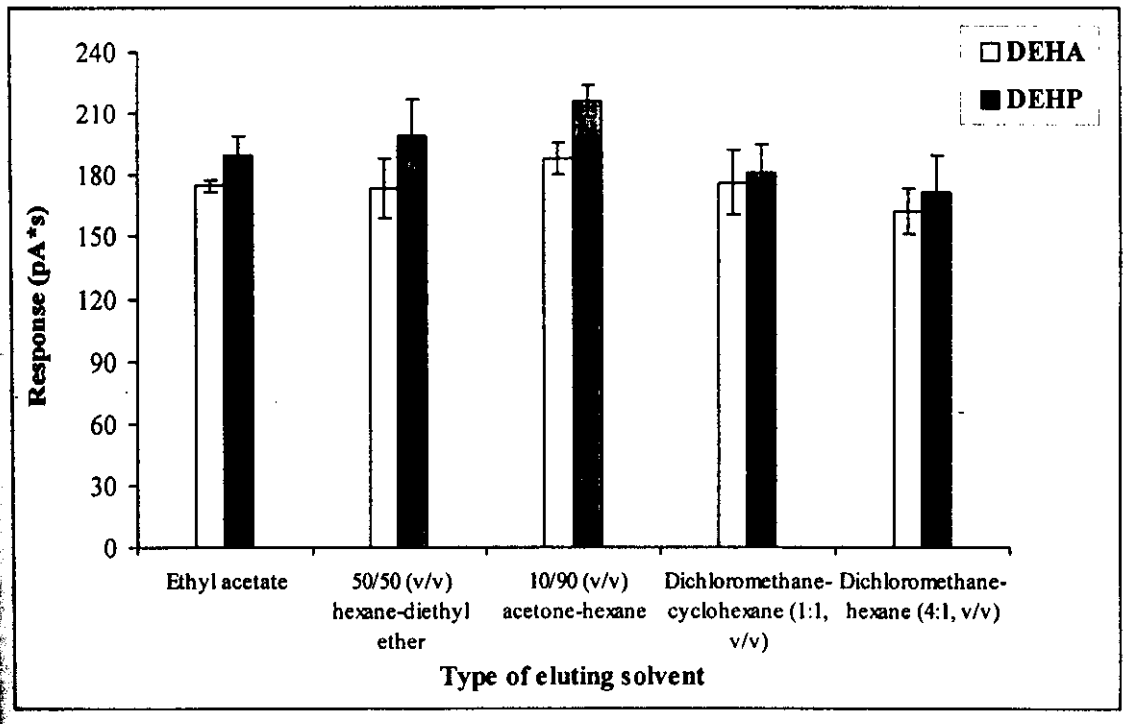

Figure 32 Responses of DEHP and DEHA at various eluting solvents

3.4.2.4 Volume of eluting solvent

For the elution step, it is important to consider the volume of solvent because it would affect the extraction efficiency. In SPE, unlike liquid-liquid extraction (LLE), it does not require large volume of organic solvents, but if the volume is too small, it may not be sufficient to elute the analyte on the sorbent. Therefore, it was necessary to optimize the volume of eluting solvent. In this study, Florisil® cartridge was used to concentrate and cleanup. The adsorbed DEHP and DEHA were eluted by using acetone-hexane (10/90, v/v) and the results are shown in Table 29 and Figure 33. The volume of 5 mL gave the highest response for both DEHP and DEHA and this was selected to be the appropriate solvent volume to elute DEHP and DEHA from Florisil® cartridges.

Table 29 Effect of volume of eluting solvent on the response of DEHP and DEHA

Volume of eluting solvent (mL)	Response (pA*s)*	
	DEHA	DEHP
1	140±15	127±8
3	154±15	161±24
5	164±13	174±16
7	155±22	162±19
9	145±12	155±20

*5 replications, RSD ≤ 15%

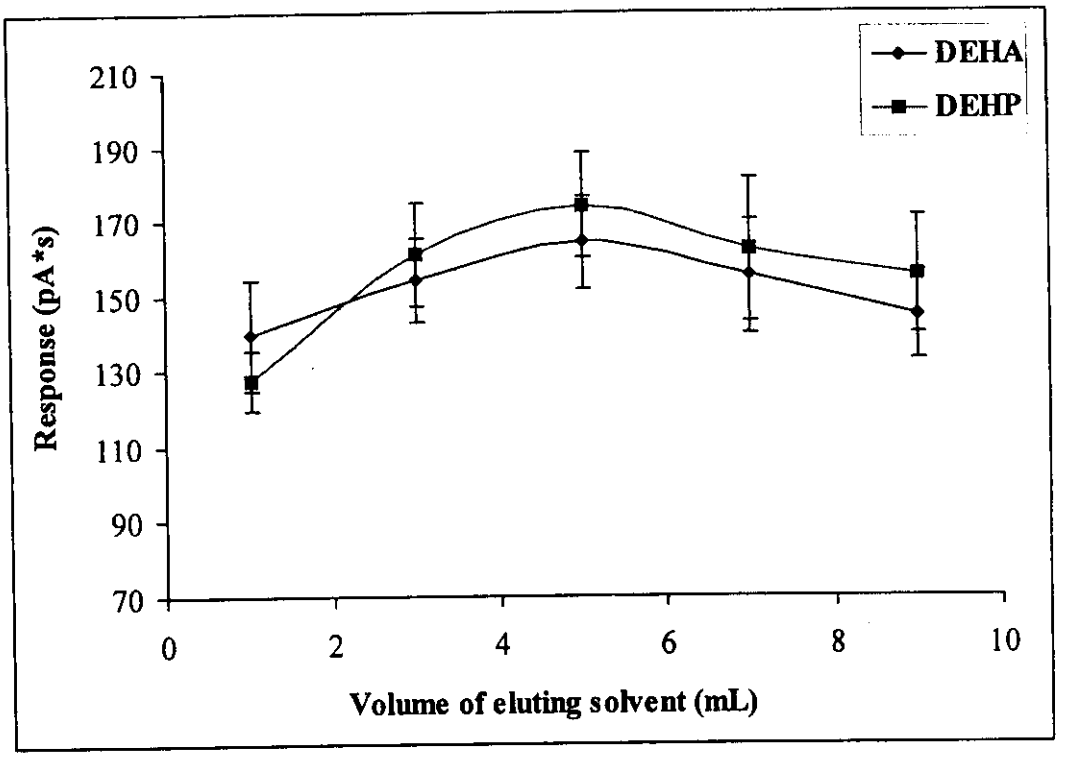

Figure 33 Responses of DEHP and DEHA at various volumes of eluting solvent

3.4.2.5 Flow rate of eluting solvent

In elution step, analyte molecules can still be retained on the sorbent. When the flow rate is either too high or low, it will give low reponse and lead to poor recovery. When developing a method, it is of great value to determine the fastest flow rate possible that still yields good recovery. This typically ensures enough residence time for the eluting solvent to sufficiently interact with the sorbent. By properly controlling and optimizing flow rate parameters for an SPE procedure, one can increase the ruggedness of an SPE method eliminating one potential cause often attributed to low and variable recoveries (Sigma-Aldrich, 2005). Table 30 and Figure 34 show the responses at different flow rates. A flow rate of 5 mL min^{-1} provided the highest response. Therefore, 5 mL min^{-1} was selected as the optimum flow rate.

Table 30 Effect of flow rate of eluting solvent on the response of DEHA and DEHP

Flow rate (mL min^{-1})	Response (pA*s)*	
	DEHA	DEHP
1	160±6	156±6
3	164±9	177±8
5	177±10	197±10
7	169±11	172±14

*5 replications, RSD ≤ 8%

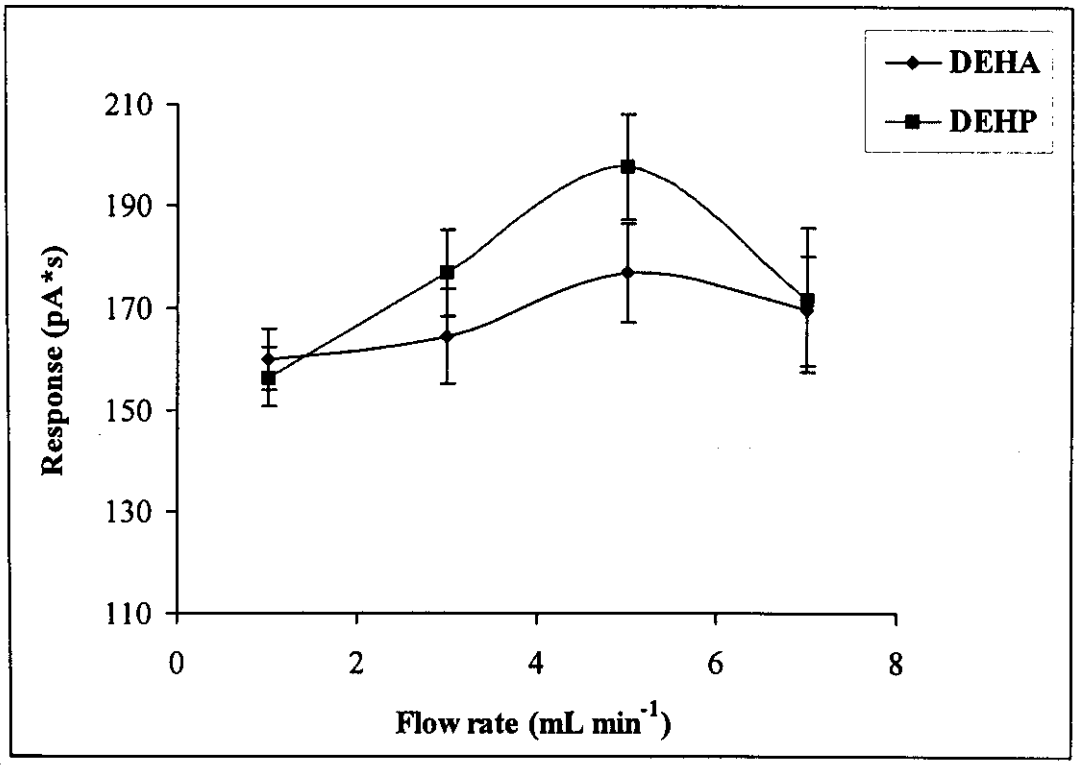

Figure 34 Responses of DEHP and DEHA at various eluting solvent flow rates

The optimum conditions of sample preparation procedure for the analysis of phthalate and adipate esters in packaged food with GC-FID using HP-5MS capillary column are summarized in Table 31. The chromatograms obtained by these sample preparation conditions (Figure 35) gave baseline resolution.

Table 31 Optimum conditions of sample preparation

Parameters	Optimum values
Ultrasonic extraction:	
Extraction time	90 minutes
Extraction solvent	dichloromethane-cyclohexane 1:1 (v/v)
Volume of extraction solvent	20 mL
Solid phase extraction:	
Sample flow rate	3 mL min^{-1}
Drying time	6 minutes
Type of eluting solvent	acetone-hexane 10/90 (v/v)
Volume of eluting solvent	5 mL
Flow rate of eluting solvent	5 mL min^{-1}

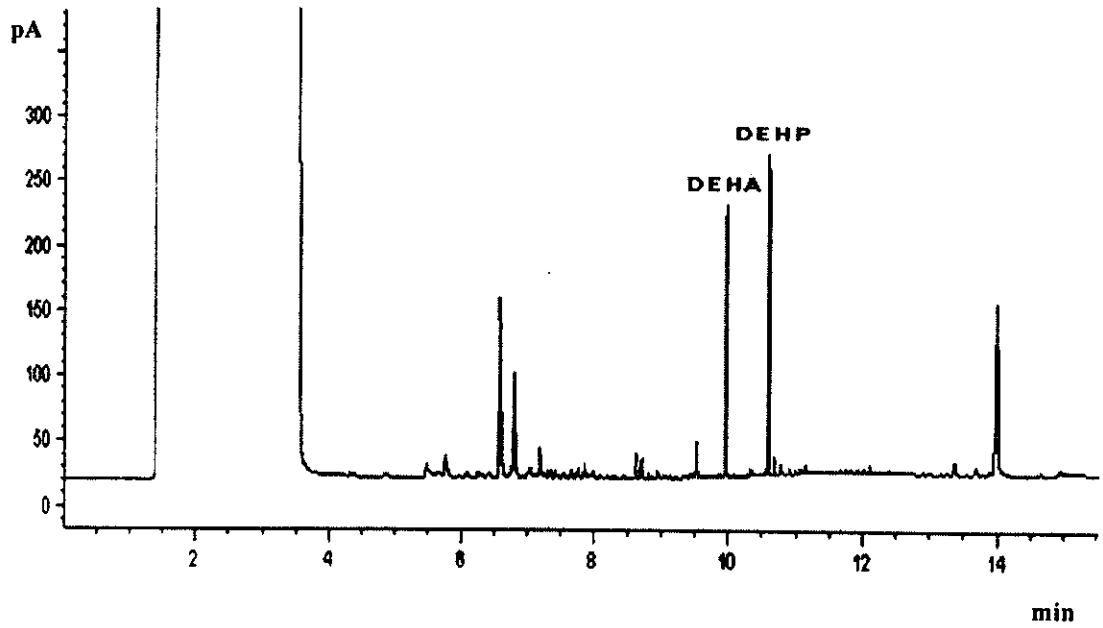

Figure 35 GC-FID Chromatograms of DEHA and DEHP from spiked curry pastes sample at optimum conditions of sample preparation

3.5 Matrix Interference

The matrix had a significant effect on the sample preparation process since curry paste sample consists of various components and the matrix would differ from type to type and this could interfere with the interest analytes. If the analyst are not awared of these interferences, it can lead to either a suppression or enhancement of the sample signal compared to the calibrant signal for the same analytes. Interference would usually affect the slope of the calibration curve, so that it will be different from the slope of the analyte of interest, so the slope of the calibration curve in the method of additions may affect the linearity of the curve. This effect has the potential to indicate the possible present of a hidden interference (Eurachem, 1998). The investigation was carried out in 2.10. Five types of curry paste samples *i.e.*, Sour yellow, Red, Green, Masman and Panang curry pastes were used to study the matix interference. The matrix match calibration curve was used for this study by spiking the various amounts of known DEHP-DEHA standard solution into the sample as described in 2.10. The optimum conditions were set for determining the spiked sample and standard DEHP and DEHP. Figure 36 shows an example of GC-FID

chromatograms of spiked Sour yellow curry paste sample. From the chromatograms, it can be seen that matrix interference are presented but chromatographically separated from DEHP and DEHA. The presence of an interference can be confirmed by observing the slope of standard curve and the spiked sample calibration (matrix match calibration curve) and determining whether or not it is parallel to the standard curve because of the slope of the calibration curve will affect the linearity of the curve. If the matrix effect not present, the instrument responses consider from both the slope of the standard curve and the spiked sample calibration should be the same. (Eurachem, 1998; Roper *et al.*, 2001). The results are shown in Tables 32-33 and Figures 37-38. When the standard and the spiked sample calibration curves were compared, the two sets of data do not have parallel regression lines, which indicates interference from the packaged food composite on phthalate and adipate esters analysis. To confirm the matrix interference of this method, the statistic test was use as described in 3.6.

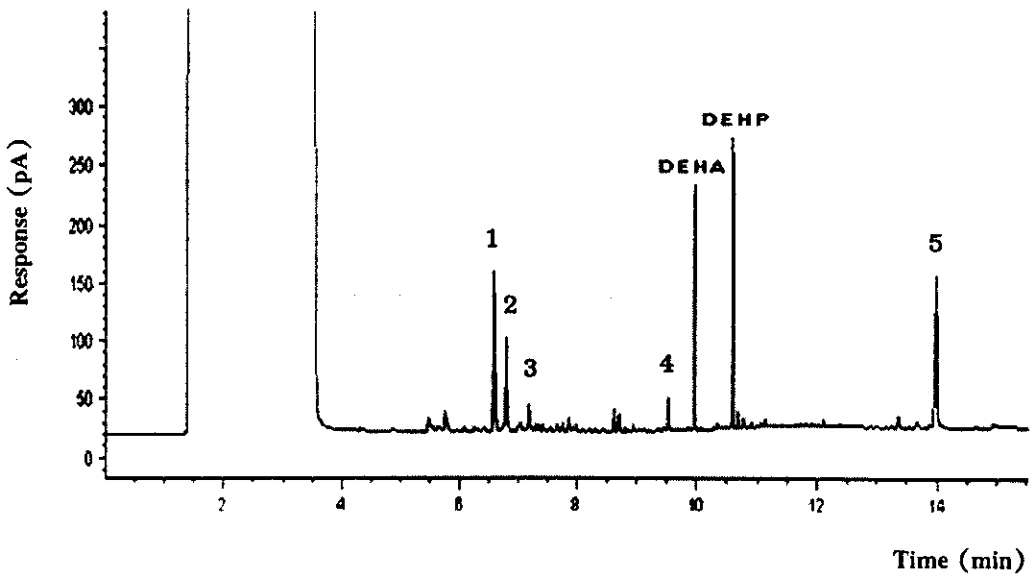

Figure 36 GC-FID Chromatograms of DEHA and DEHP from spiked curry paste sample with some interference peaks (peak No. 1, 2, 3, 4 and 5)

Table 32 Effect of matrix on the responses of DEHA in curry paste samples

Concentration of DEHA ($\mu g\ mL^{-1}$)	Response (pA*s)*± SD					
	Standard DEHA	Sour yellow curry paste	Red curry paste	Green curry paste	Masman curry paste	Panang curry paste
0.05	26.1±0.4	19.5±0.9	17.2±0.5	23.7±1.9	16.8±1.5	13.1±1.4
1.0	40.3±1.5	31.5±3.2	28.1±2.7	35.7±2.2	24.1±2.0	24.2±2.4
5.0	197.7±4.6	175.8±11.8	181.0±14.0	163.4±5.4	123.6±5.5	126.2±6.6
10.0	336.3±4.5	295.9±11.7	197.3±9.6	277.5±19.3	254.9±11.4	226.6±10.8

*5 replications, RSD ≤ 10%

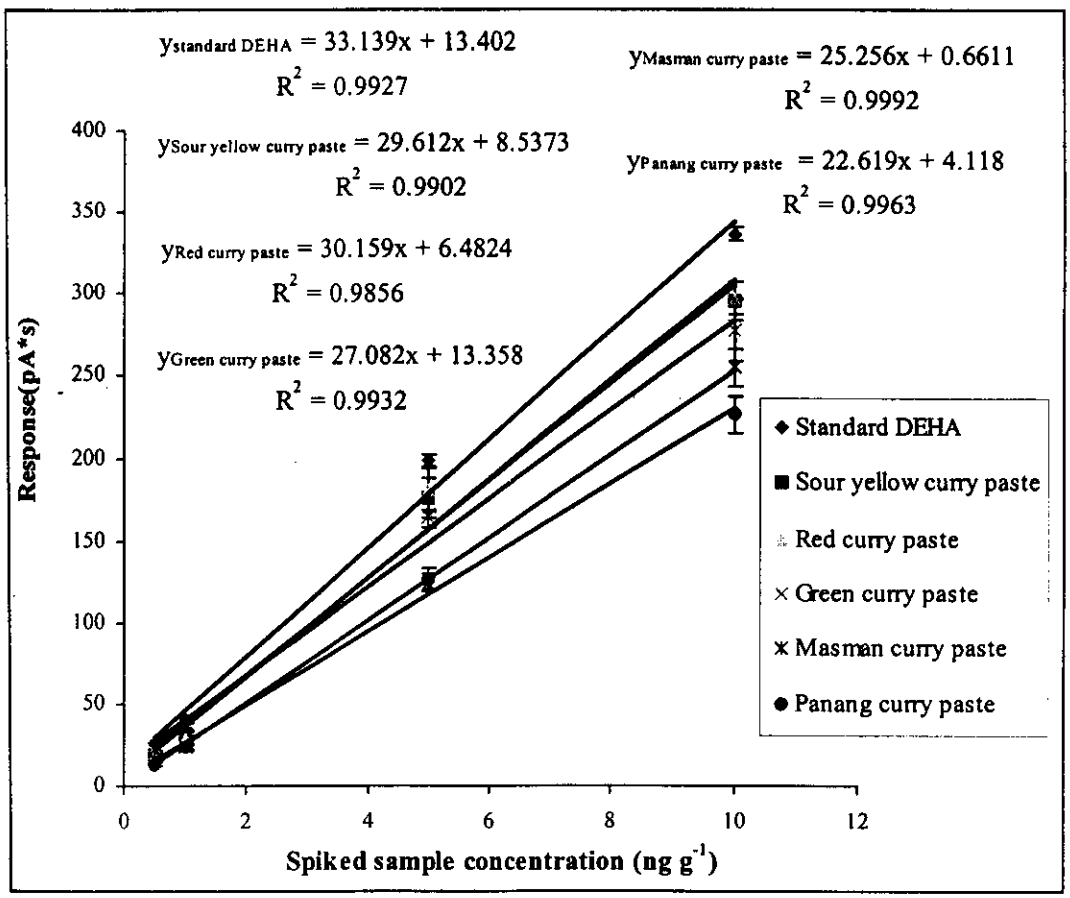

Figure 37 Matrix match calibration curve of DEHA of curry paste samples

Table 33 Effect of matrix on the responses of DEHP in curry paste samples

Concentration of DEHP ($\mu g\ mL^{-1}$)	Response (pA*s)*± SD					
	Standard DEHA	Sour yellow curry paste	Red curry paste	Green curry paste	Masman curry paste	Panang curry paste
0.05	22.8±1.0	17.5±0.6	21.1±1.9	14.7±0.9	7.5±0.4	12.0±0.9
1.0	35.9±0.9	20.9±0.6	29.3±2.0	26.4±2.6	14.8±1.4	20.2±0.7
5.0	173.3±5.6	136.1±12.5	160.8±13.5	131.1±4.1	97.0±7.1	110.8±6.5
10.0	296.6±3.9	247.3±14.4	261.3±12.6	232.0±18.0	204.5±5.3	195.6±9.8

*5 replications, RSD ≤ 10%

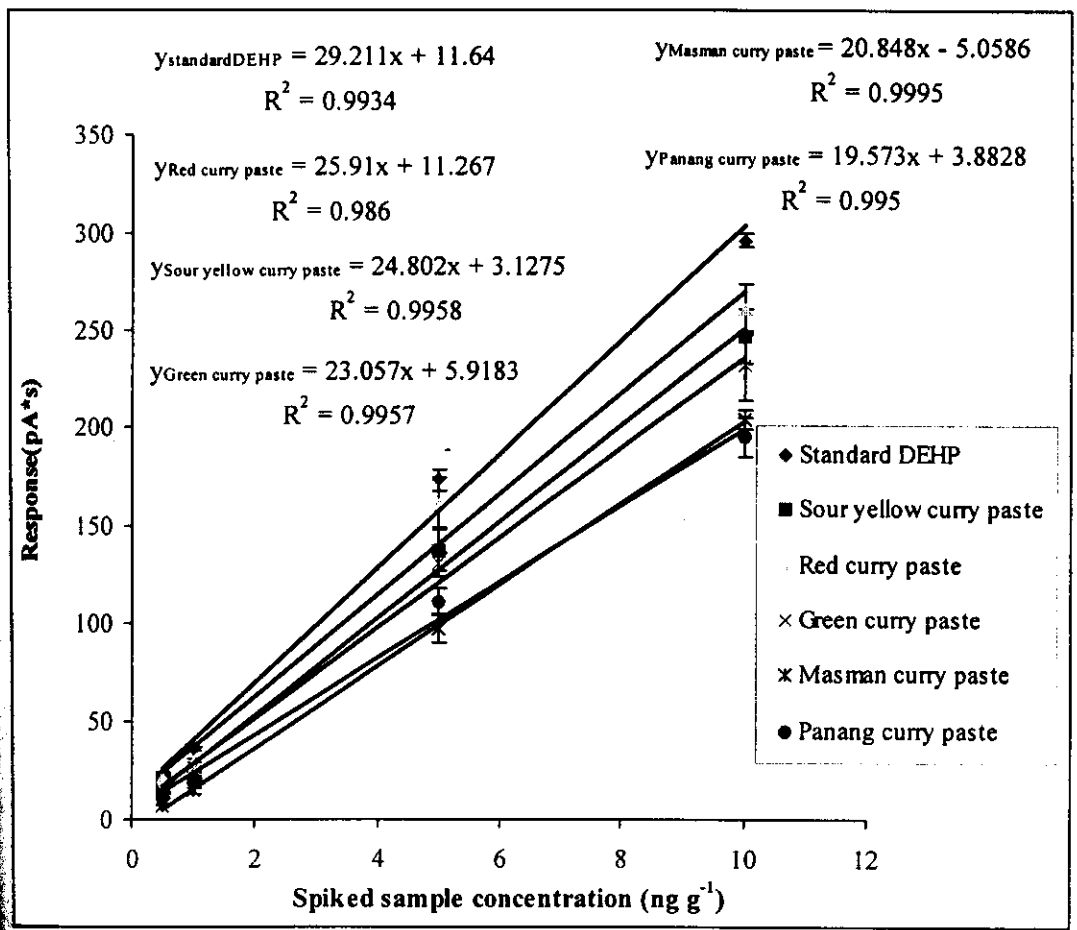

Figure 38 Matrix match calibration curve of DEHP of curry paste samples

3.6 Comparison between the slope of standard and matrix match calibration curve

To confirm the matrix interference a statistical test known as a significance test was employed to test whether the difference between the two results is significant, or whether it can be accounted for merely by random variations. Significance tests are widely used in the evaluation of experimental results. In this experiment the use of different concentrations is a controlled factor since the concentrations are chosen by the experimenter. The curve on which the experiment is performed introduces uncontrolled variation. The slopes of standard curve and matrix match calibration curve were tested using two-way ANOVA (analysis of variance)

In making a significance test, the truth of a hypothesis which is known as a null hypothesis, denoted by H_0 that the interaction of both slope is not significant and an alternative hypothesis (H_1) that the interaction of slope is significant. If the *P* value is less than α (level of significance), then the null hypothesis was rejected at the significant level (Miller and Miller, 2000). The *P* value was calculated by R software (R development Core Team, 2006).

The results from significant test for the comparison of standard curve and matrix match calibration curve are shown in Tables 34-36.

Where *Df* is the degree of freedom, it refers to the number of independent deviation,

Df = *n*-1 (*n* is the number of concentration = 4-1 = 3)

Sum Sq is the sum of squares, it refers to an interim quantity used in the calculation of an estimate of the population variance

Mean Sq is the mean square, it refers to a sum of squared terms devided by the number of degrees of freedom

F is the ratio of two sample variances, *i.e.*, the ratio of the squares of the standard deviations, $\frac{s_1^2}{s_2^2}$

P: Probability

Table 34 Statistical values for the comparison between the slope for DEHP standard curve and matrix match calibration curve of various curry paste samples using ANOVA by R software

Matrix (type of curry paste)	*Df*	Sum Sq	Mean Sq	*F*	*P*
Sour yellow	3	2538	846	11.605	$2.622\times10^{-5***}$
Red	3	1335	445	12.095	$1.868\times10^{-5***}$
Green	3	13111	4370	229.72	$2.2\times10^{-16***}$
Masman	3	10719	3573	312.58	$2.2\times10^{-16***}$
Panang	3	13111	4370	229.72	$2.2\times10^{-16***}$

Table 35 Statistical values for the comparison between the slope for DEHA standard curve and matrix match calibration curve of various curry paste samples using ANOVA by R software

Matrix (type of curry paste)	*Df*	Sum Sq	Mean Sq	*F*	*P*
Sour yellow	3	1193	398	5.0371	$5.7\times10^{-3**}$
Red	3	855	285	3.8991	$1.7\times10^{-2*}$
Green	3	4426	1475	18.505	$3.8\times10^{-7***}$
Masman	3	14736	4912	102.33	$2.2\times10^{-6***}$
Panang	3	14736	4912	102.33	$2.2\times10^{-6***}$

Significant codes: '*' (α = 0.01), '**' (α = 0.05), '***' (α = 0.001)

Table 36 Level of significance (*P* value) from ANOVA for the comparison between the slope for standard curve and matrix match calibration curve of various curry paste samples

Matrix	*P*	
	DEHP	DEHA
Sour yellow	0.001	0.01
Red	0.001	0.05
Green	0.001	0.001
Masman	0.001	0.001
Panang	0.001	0.001

From the result of the interaction between responses and concentrations in standard and matrix groups, it can be concluded that the slope of regression line in each group of standard curve and matrix match calibration curve were significantly different at various levels of significance as shown in Table 36. Thus, the interference is present. If an interference is present, the matrix match calibration curve may allow an accurate determination of the unknown concentration (Eurachem, 1998; Roper *et al.*, 2001).

3.7 Method validation

Method validation is performed to create confidence in the analysis procedures. It provides an assurance of reliability during normal use (Swartz, 1997). To ensure the reliability of the results, method validation was evaluated by using standards, spiked samples, reagent and method blanks. The results of the studies are described in 3.7.1- 3.7.4.

3.7.1 Recovery

In order to evaluate the sample preparation procedure, high recovery of the analytes from the matrix is a desirable outcome and is, therefore, an important characteristic of the extraction procedure. Recovery of the phthalate and adipate esters in packaged food samples were tested by spiking known amounts of DEHP-DEHA standard solution into curry paste sample at concentration level of 0.5 and 5.0 μg mL^{-1}, as decribed in 2.11.1. The responses obtained from a spiked curry paste sample and from DEHP-DEHA standard solution were compared. The results are shown in Tables 37 and 38, acceptable recoveries (70-120% by EPA method 8061, 1996) were obtained, ranged from 93 to 100% and 88 to 99% for DEHP and DEHA at spiked concentration 0.5 and 5.0 μg mL^{-1} respectively. These values were closed to those obtained by Tsumura and coworkers (2002) that applied Florisil® and Bondesil PSA® dual layer columns to cleanup phthalates, adipates, citrate and some other plasticizer from retail food where the recoveries were in the range of 95-106%. The recovery in this study was better than obtained by Casajuana and Lacorte (2004) who applied Florisil® to extract di (2-ethylhexyl) phthalate (DEHP) and the recovery was 86%. The results from this study, indicate that the ultrasonic extraction method followed by solid phase extraction can be used for the extraction of phthalate and adipate esters from curry paste sample with high percentage recoveries.

Table 37 Percentage recovery of DEHP and DEHA of various curry paste samples at spiked concentration of 0.5 μg mL^{-1}

Type of curry paste samples	Recovery* (%) ± SD	
	DEHP	DEHA
Sour yellow curry paste	100±2	99±2
Red curry paste	100±6	95±2
Green curry paste	93±3	93±6
Masman curry paste	97±6	94±1
Panang curry paste	97±6	95±1

*5 replications, RSD < 7%

Table 38 Percentage recovery of DEHP and DEHA of various curry paste samples at spiked concentration of 5.0 μg mL^{-1}

Type of curry paste samples	Recovery* (%) ± SD	
	DEHP	DEHA
Sour yellow curry paste	97±2	95±2
Red curry paste	91±1	88±1
Green curry paste	99±6	98±6
Masman curry paste	98±8	95±9
Panang curry paste	94±4	94±2

*5 replications, RSD < 10%

3.7.2 Method detection limit (MDL)

Five types of curry paste samples were spiked with DEHP-DEHA standard solution to make final concentrations in the range of 27 to 250 μg mL^{-1} and

analyzed together with nonspiked (blank) samples. The analyte peak areas were corrected for any interference found in blank samples by substracting the peak area of the interference in blanks from the analyte peak areas in spiked samples. The method detection limit (MDL) of this method was calculated by a signal to noise ratio (S/N) more than 3 were between 27 and 30 ng mL^{-1} (Table 39).

Table 39 The method detection limit of DEHA and DEHP analysis using ultrasonic extraction follow by SPE sample preparation methods for various curry paste samples

Type of curry paste samples	Method detection limit* (ng mL^{-1})	
	DEHA	DEHP
Sour yellow curry paste	30	27
Red curry paste	30	30
Green curry paste	30	27
Masman curry paste	30	27
Panang curry paste	30	27

*5 replications, RSD < 10%

3.7.3 Limit of quantitation (LOQ)

The limit of quantitation (LOQ) is regarded as the lower limit for precise quantitative measurements (Corley, 2002). The LOQ was calculated as the analyte concentration giving a signal to noise ratio of 10 times. The LOQs of DEHP and DEHA are shown in Table 40. The LOQ ranged from 90 to 100 ng mL^{-1}.

Table 40 Limit of quantitation (LOQ) of DEHA and DEHP at various curry paste samples with S/N ≥10

Curry paste samples	Method detection limit* (ng mL^{-1})	
	DEHA	DEHP
Sour yellow curry paste	100	90
Red curry paste	100	90
Green curry paste	100	100
Masman curry paste	100	90
Panang curry paste	100	90

*5 replications, RSD < 10%

3.7.4 Precision

Precision is the closeness of agreement between independent test results obtained under stipulated conditions. It is a measure of how close results are to one another, and is usually expressed as a relative standard deviation (RSD), which describe the spread of results (Eurachem Guide, 1998). It is generally dependent on analyte concentration. Repeatability is a type of precision relating to measurements made under repeatable conditions, *i.e.*, same method, same material, same operator and same laboratory (Eurachem, 2002). The repeatability of the whole analytical procedure is an evaluation of the overall extraction purification and analysis procedure (Sablayrolles *et al.*, 2005). It is calculated on the basis of five replications of five different curry paste samples. In this study, five types of curry paste samples were evaluated at two spiking level of 0.5 and 5.0 μg mL^{-1} in each curry paste sample followed by sample preparation procedure before injected into GC-FID system at optimum conditions as described in 2.11.4. Five replicate analyses were performed at each concentration. The results are shown in Table 41 and 42. They show good precision with relative standard deviations (RSD) lower than 8 and 10% at spiked concentration 0.5 and 5.0 μg mL^{-1} for both for DEHP and DEHA. These values were

better than the recommendation by EPA method 8000 (1996) that accepted RSD less than or equal to 20%. Method 8000 from USEPA SW846 provides guidance on analytical chromatography and lists quality control requirements common to all the gas chromatographic method. This is typical for gas chromatographic detector other than the mass spectrometer.

Table 41 Precision of curry paste samples analysis for DEHP at spiked concentration of 0.5 and 5.0 $\mu g\ mL^{-1}$

Type of curry paste samples	**Precision*, RSD (%)**	
	0.05 $\mu g\ mL^{-1}$	**5.0 $\mu g\ mL^{-1}$**
Sour yellow curry paste	1.8	2.2
Red curry paste	4.8	0.3
Green curry paste	3.7	5.7
Masman curry paste	6.5	7.9
Panang curry paste	5.7	3.7

*5 replications

Table 42 Precision of curry paste samples analysis for DEHA at spiked concentration of 0.5 and 5.0 μg mL^{-1}

Type of curry paste samples	Precision*, RSD (%)	
	0.05 μg mL^{-1}	5.0 μg mL^{-1}
Sour yellow curry paste	2.6	2.4
Red curry paste	4.9	1.4
Green curry paste	6.7	6.1
Masman curry paste	5.4	9.7
Panang curry paste	0.6	1.8

*5 replications

3.8 Qualitative and quantitative analysis of phthalate and adipate esters in food

3.8.1 Qualitative analysis

The optimum conditions of GC-FID were used to analyse phthalate and adipate esters in food samples. For qualitative analysis, the most frequently used technique for the tentative identification of an eluted component is based on the retention times (t_R) of the eluted component and those of known standard solution (Gudzinowicz, 1967). The average t_R of DEHA and DEHP were 10.02±0.05 and 10.60±0.05 minutes, respectively.

3.8.2 Quantitative Analysis

The quantitative analysis of DEHP and DEHA were done by considering the response, *i.e.*, peak area, that was related to the concentration of the analytes. Five types of curry paste samples *i.e.*, Sour yellow, Red, Green, Masman and Panang curry pastes were sampling from supermarkets. All curry paste samples were extracted and cleanup by ultrasonic extraction and solid phase extraction (SPE)

using Florisil® cartridges. The samples were analyzed by GC-FID using optimum conditions and the representative chromatogram of the five types of curry paste sample are shown in Figures 39-43. DEHP and DEHA concentrations in food in contact with plastic films were evaluated by using the matrix match calibration curve, as described in 2.12.2.1 to quantify phthalate and adipate esters in packaged food. The results are shown in Table 43 showed that the concentrations of DEHP in all curry paste samples were in the range from 0.12 to 0.61 μg g^{-1}. For DEHA, it could not be detected by matrix match calibration curve. It is possible that the amount of DEHA was lower than the method detection limit (MDL). Therefore, the confirmation was carried out by standard addition method as described in 2.12.2.2. The results for standard addition method are shown in Tables 44-53 and Figures 44-54.

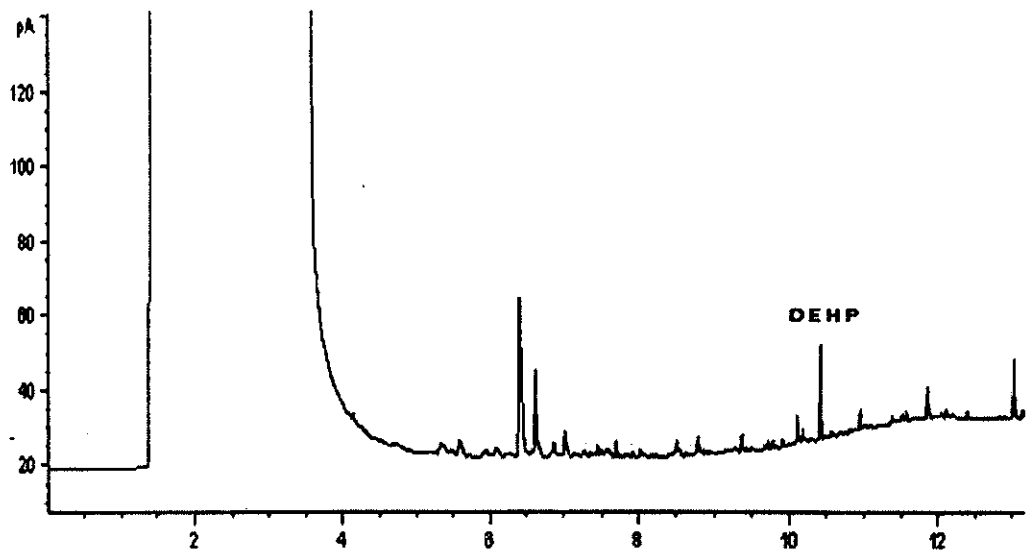

Figure 39 GC-FID Chromatogram of DEHP from Sour curry paste sample

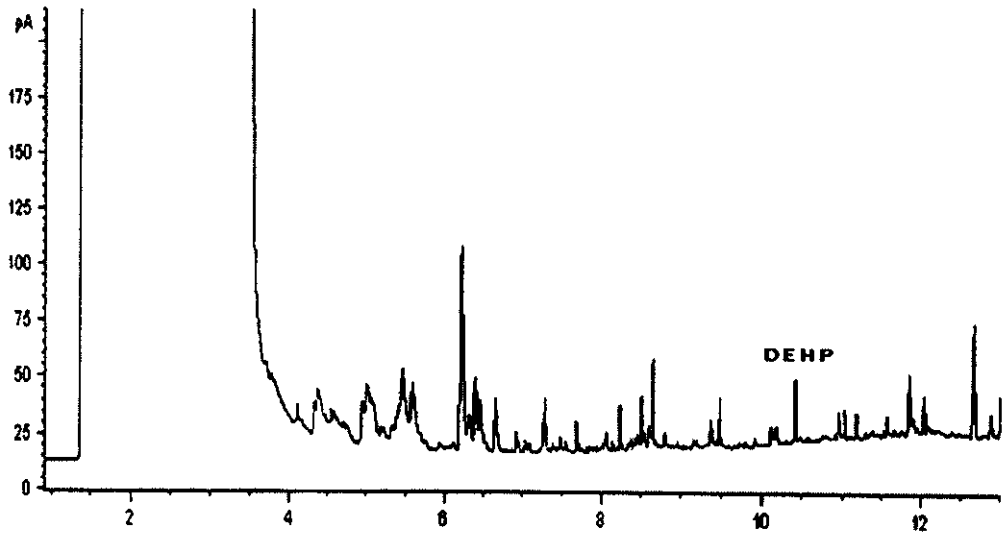

Figure 40 GC-FID Chromatogram of DEHP from Red curry paste sample

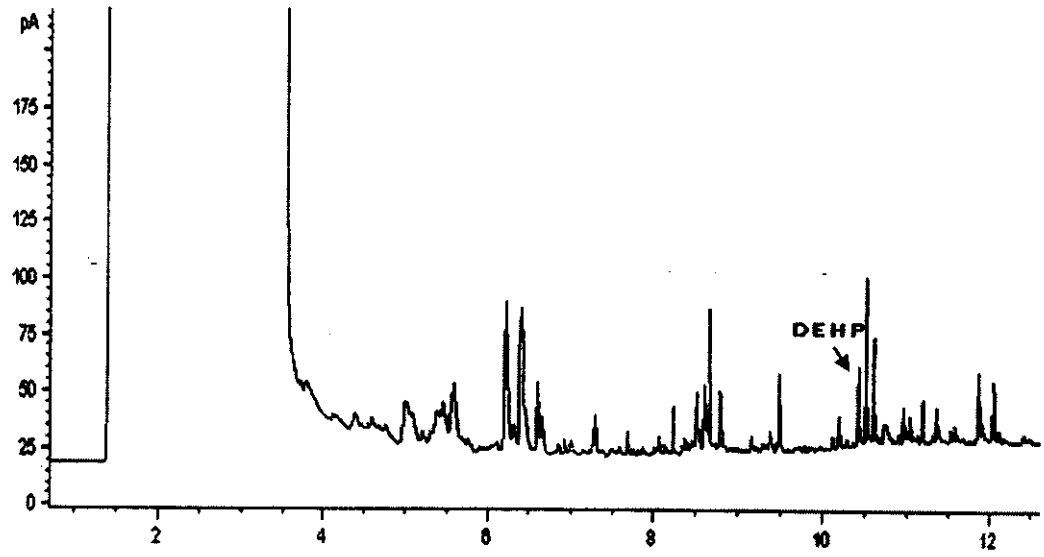

Figure 41 GC-FID Chromatogram of DEHP from Green curry paste sample

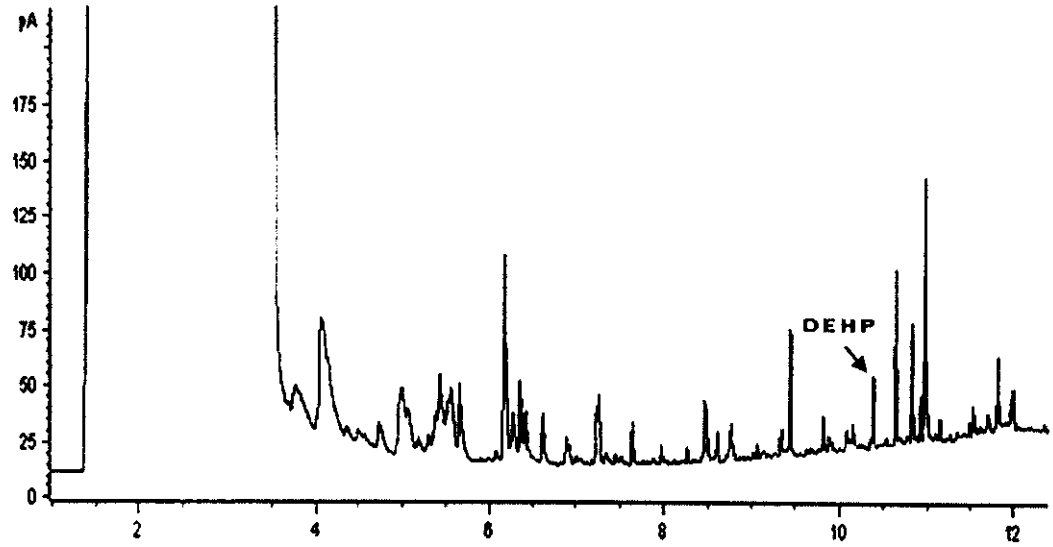

Figure 42 GC-FID Chromatogram of DEHP from Masman curry paste sample

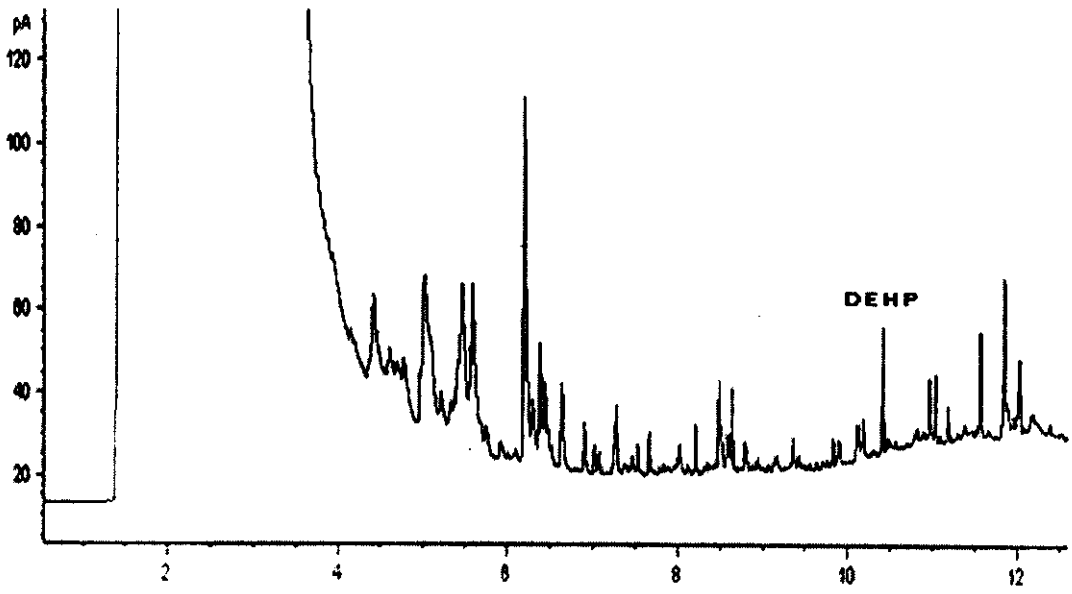

Figure 43 GC-FID Chromatogram of DEHP from Panang curry paste sample

Table 43 The DEHP concentrations of each curry paste sample determined by matrix match calibration curves

Types of sample	Amount of DEHP ($\mu g\ g^{-1}$)*	
	Sample No. 1	**Sample No. 2**
Sour yellow curry paste	0.58±0.02	0.61±0.02
Red curry paste	0.36±0.03	0.46±0.02
Green curry paste	0.17±0.02	0.37±0.01
Masman curry paste	0.37±0.02	0.38±0.02
Panang curry paste	0.12±0.02	0.15±0.01

*5 replications, RSD $\leq$ 17%

Table 44 The results of standard addition calibration curves of DEHA in Sour yellow curry paste sample (sample No. 1) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.5±0.2
60	6.5±0.3
90	8.3±0.2
100	10.8±0.4

*5 replications, RSD < 6%

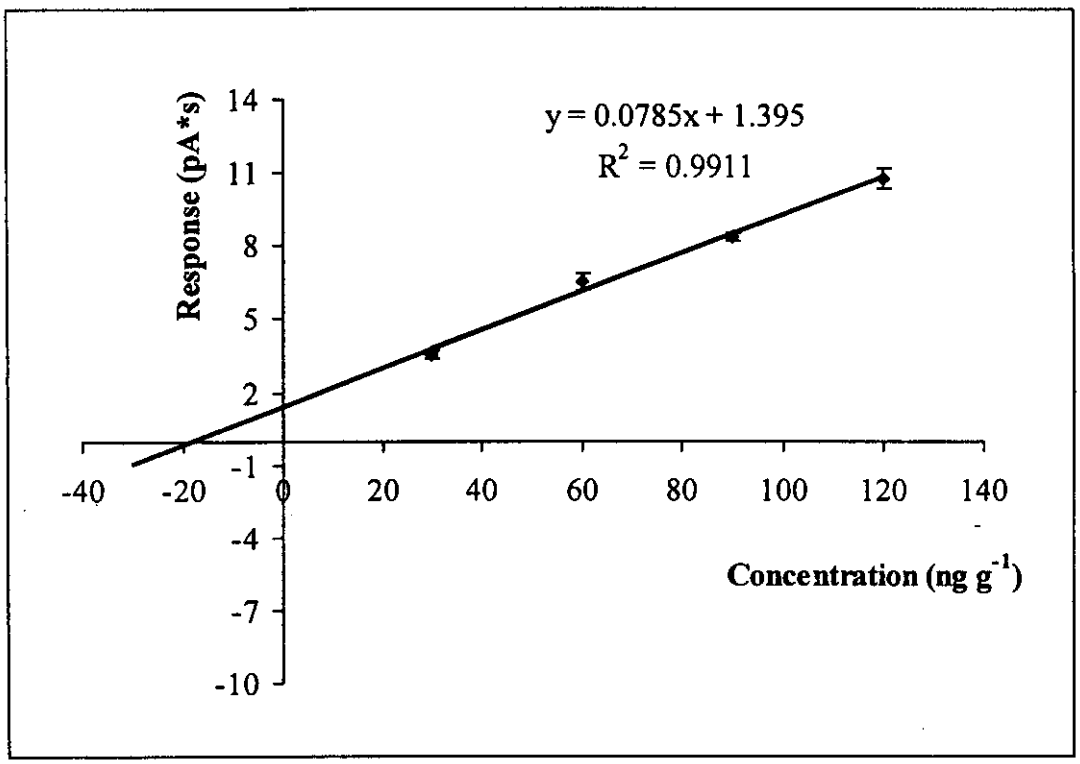

Figure 44 The standard addition calibration curve of DEHA in Sour yellow curry paste sample (sample No. 1)

Table 45 The results of standard addition calibration curves of DEHA in Sour yellow curry paste sample (sample No. 2) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.1±0.1
60	6.1±0.4
90	8.3±0.4
100	10.3±0.3

*5 replications, RSD < 8%

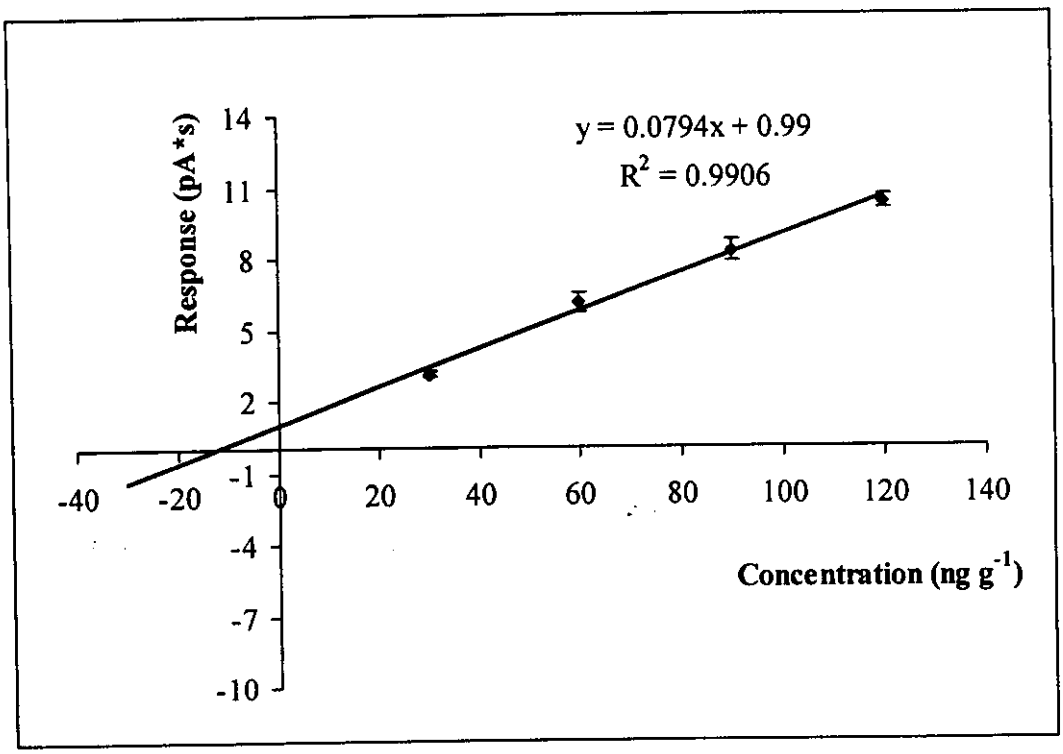

Figure 45 The standard addition calibration curve of DEHA in Sour yellow curry paste sample (sample No. 2)

Table 46 The results of standard addition calibration curves of DEHA in Red curry paste sample (sample No. 1) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.6±0.3
60	6.5±0.1
90	8.9±0.6
100	11.0±0.4

*5 replications, RSD ≤ 10%

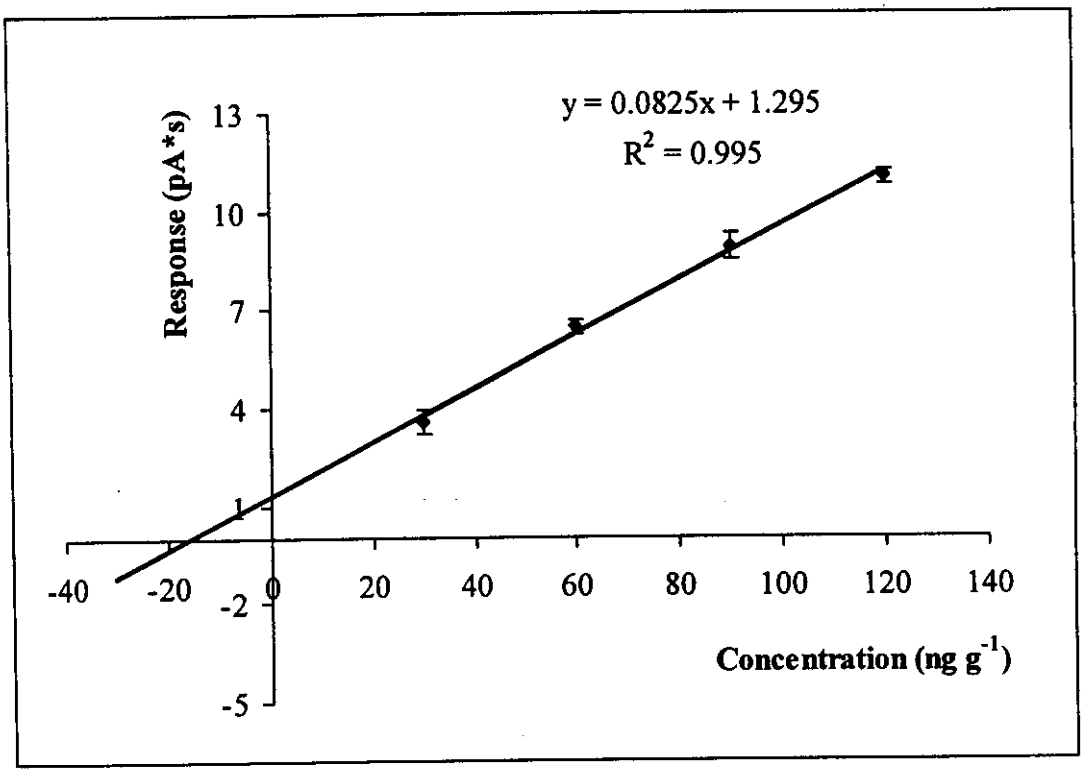

Figure 46 The standard addition calibration curve of DEHA in Red curry paste sample (sample No. 1)

Table 47 The results of standard addition calibration curves of DEHA in Red curry paste sample (sample No. 2) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.0±0.3
60	5.7±0.1
90	8.9±0.6
100	11.1±0.4

*5 replications, RSD < 10%

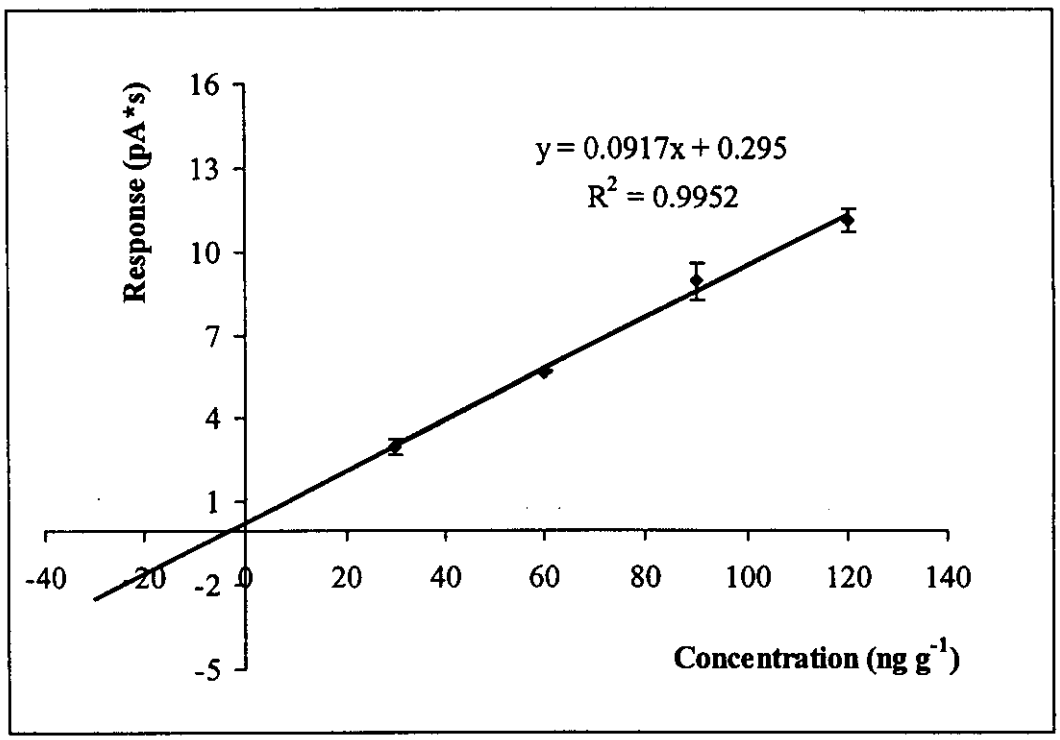

Figure 47 The standard addition calibration curve of DEHA in Red curry paste sample (sample No. 2)

Table 48 The results of standard addition calibration curves of DEHA in Green curry paste sample (sample No. 1) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.0±0.1
60	5.5±0.1
90	7.3±0.1
100	9.1±0.5

*5 replications, RSD < 6%

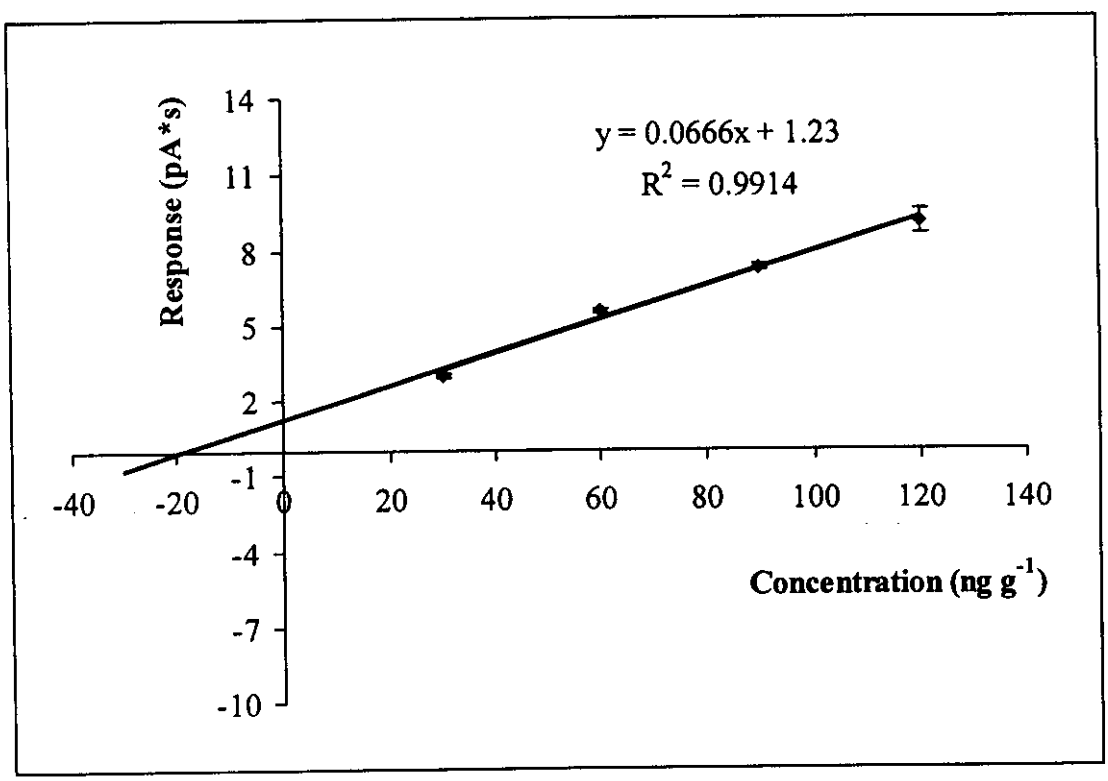

Figure 48 The standard addition calibration curve of DEHA in Green curry paste sample (sample No. 1)

Table 49 The results of standard addition calibration curves of DEHA in Green curry paste sample (sample No. 2) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.4±0.2
60	6.3±0.5
90	8.0±0.5
100	10.0±0.6

*5 replications, RSD < 6%

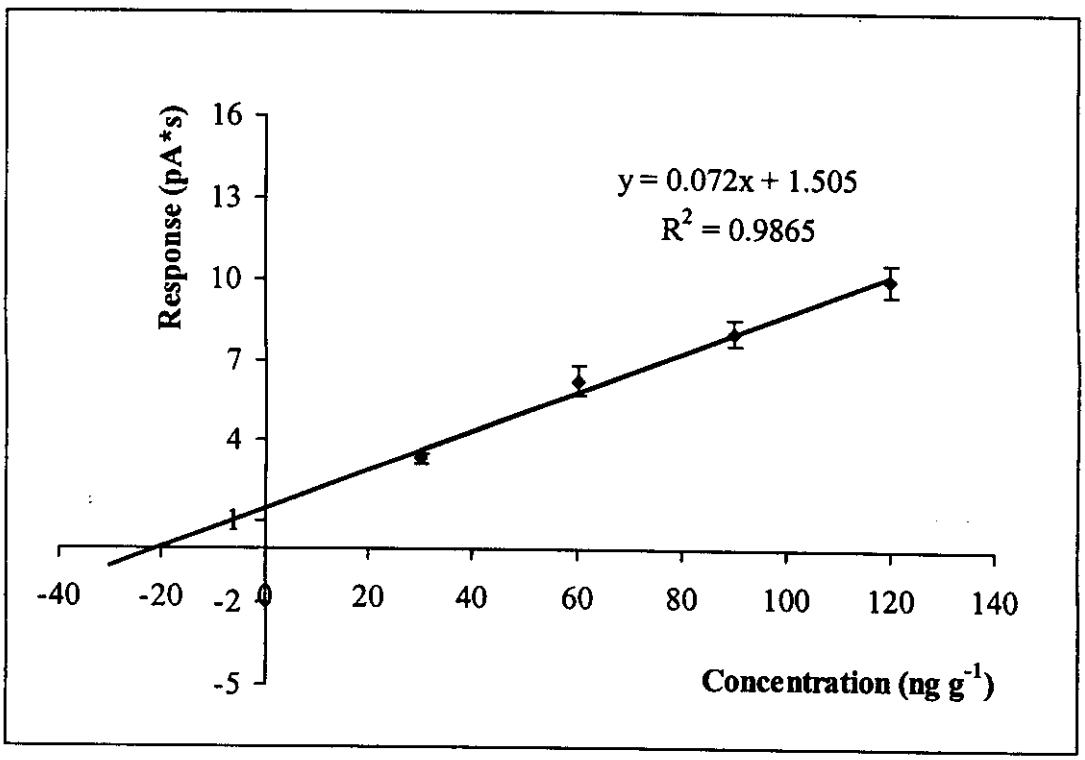

Figure 49 The standard addition calibration curve of DEHA in Green curry paste sample (sample No. 2)

Table 50 The results of standard addition calibration curves of DEHA in Masman curry paste sample (sample No. 1) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.4±0.2
60	6.5±0.2
90	8.9±0.4
100	11.2±0.3

*5 replications, RSD < 6%

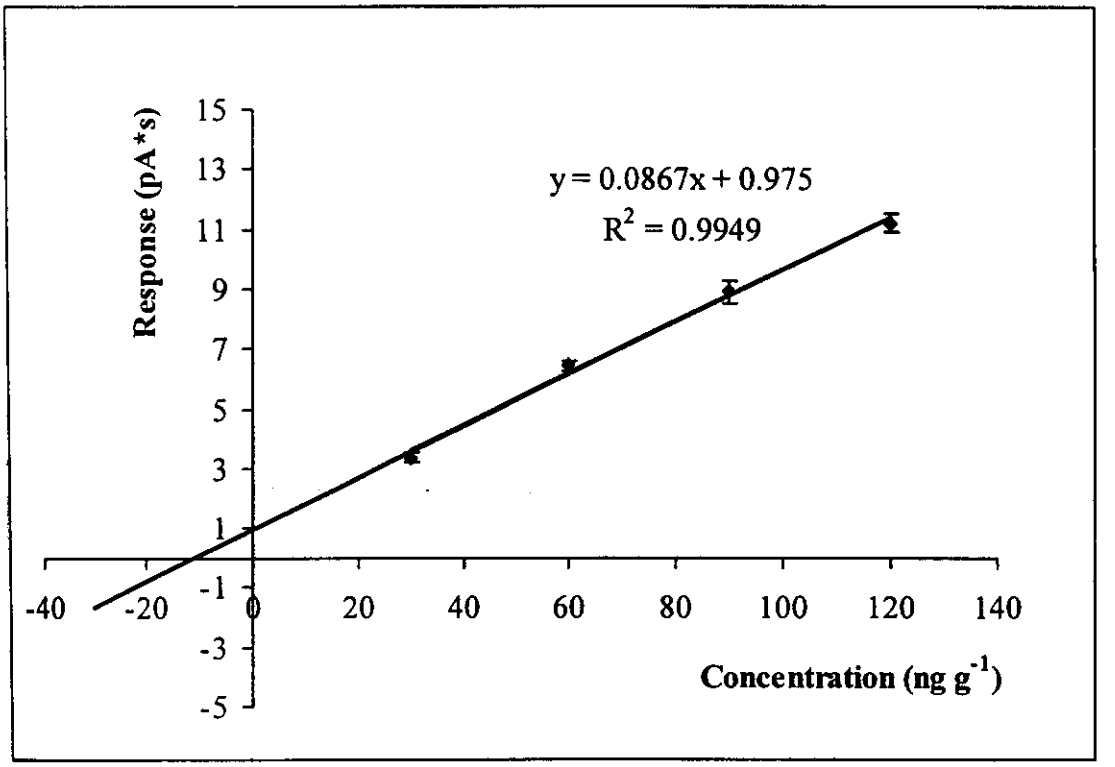

Figure 50 The standard addition calibration curve of DEHA in Masman curry paste sample (sample No. 1)

Table 51 The results of standard addition calibration curves of DEHA in Masman curry paste sample (sample No. 2) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.9±0.3
60	6.5±0.2
90	8.9±0.4
100	11.1±0.2

*5 replications, RSD < 6%

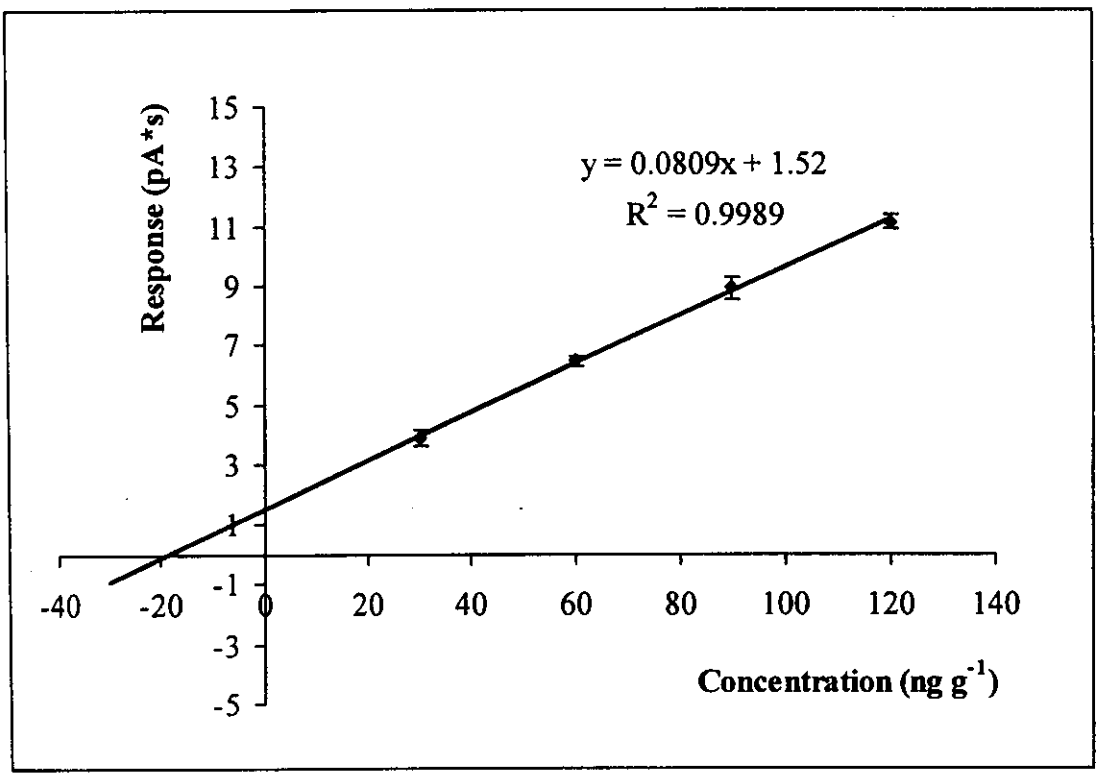

Figure 51 The standard addition calibration curve of DEHA in Masman curry paste sample (sample No. 2)

Table 52 The results of standard addition calibration curves of DEHA in Panang curry paste sample (sample No. 1) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.4±0.2
60	6.0±0.3
90	7.6±0.5
100	9.9±0.7

*5 replications, RSD ≤ 7%

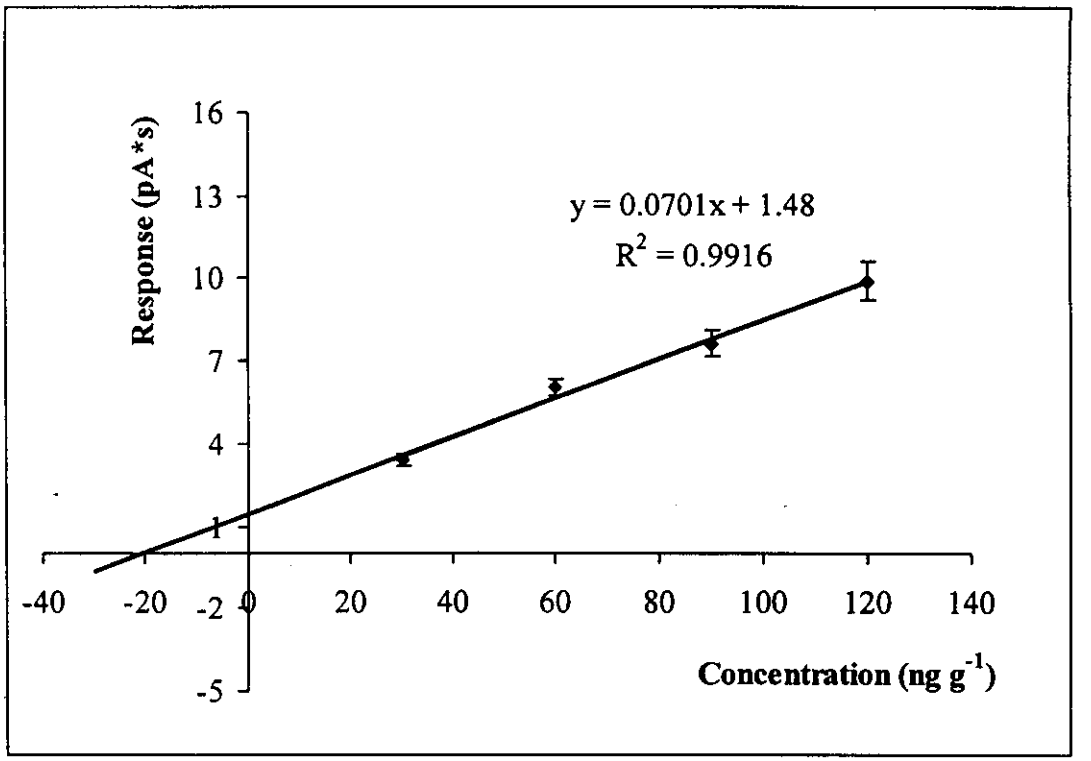

Figure 52 The standard addition calibration curve of DEHA in Panang curry paste sample (sample No. 1)

Table 53 The results of standard addition calibration curves of DEHA in Panang curry paste sample (sample No. 2) at various spiked concentration levels

Spiked concentration (ng mL^{-1})	Response (pA*s)*
30	3.0±0.1
60	5.8±0.2
90	7.8±0.3
100	9.6±0.4

*5 replications, RSD < 5%

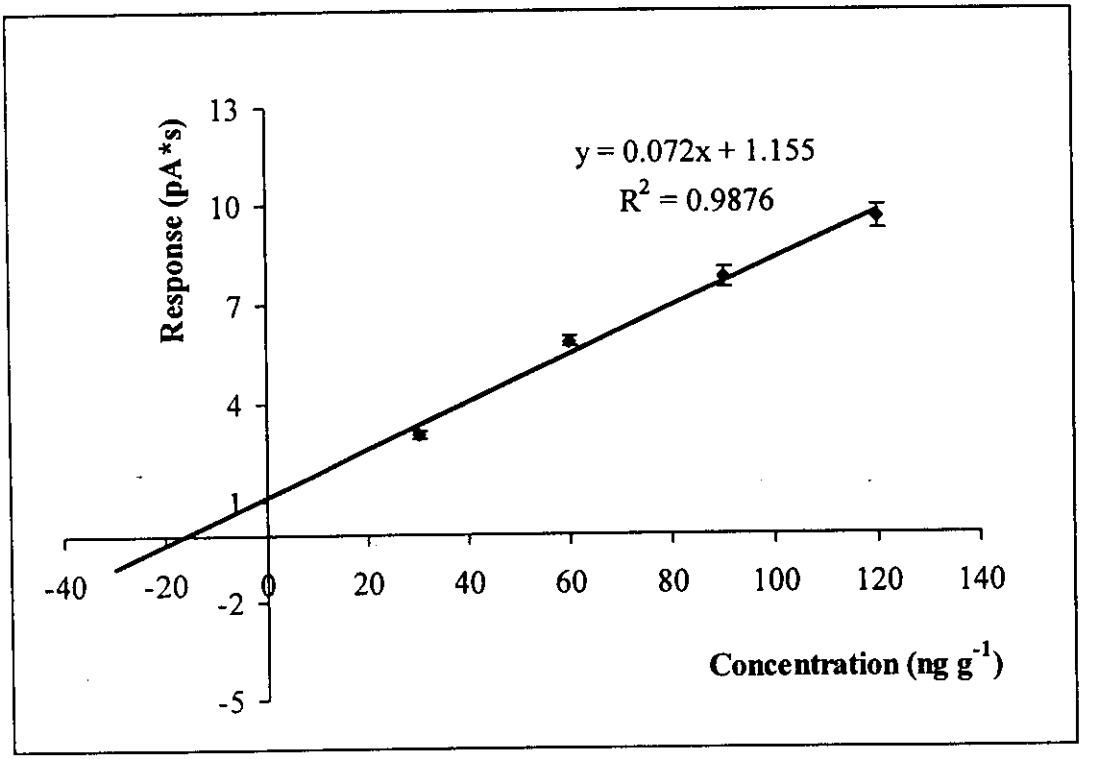

Figure 53 The standard addition calibration curve of DEHA in Panang curry paste sample (sample No. 2)

Table 54 The DEHA concentrations of each curry paste sample determined by standard addition calibration curves

Types of sample	Amount of DEHA (ng g^{-1})*	
	Sample No. 1	Sample No. 2
Sour yellow curry paste	22.2±0.1	15.9±0.2
Red curry paste	19.6±0.1	4.0±0.6
Green curry paste	23.1±0.1	26.1±0.1
Masman curry paste	14.1±0.2	23.5±0.1
Panang curry paste	26.4±0.1	20.1±0.2

The results of DEHA analysis of five types of curry paste samples determined by standard addition method are summarized in Table 54. The concentration levels of DEHA found in all 10 curry paste samples were in the range from 4.0 to 26.4 ng g^{-1}. These concentrations were calculated after measuring the response for a series of standard additions, then extrapolate to $y = 0$ to get the concentration values, C_{sa} would need to be to the response of the unknown. From $y = mx + c$, the value of x at $y = 0$ was calculated which is $C_0 = -C_{sa}(V_{total} / V_{unknown})$, where V_{total} = the total volume of the solution in each vial that are (sample +standard solution) and V_{unk} = volume of sample solution (unknown) (Zellmer, 1998). The standard deviation for each sample is calculated followed section 5.8 (the method of standard additions) in Miller and Miller (2000). The amounts of DEHP and DEHA concentrations found in five types of curry paste samples (10 samples analyzed) (as shown in Table 43 and Table 54) are summarized in Figure 54.

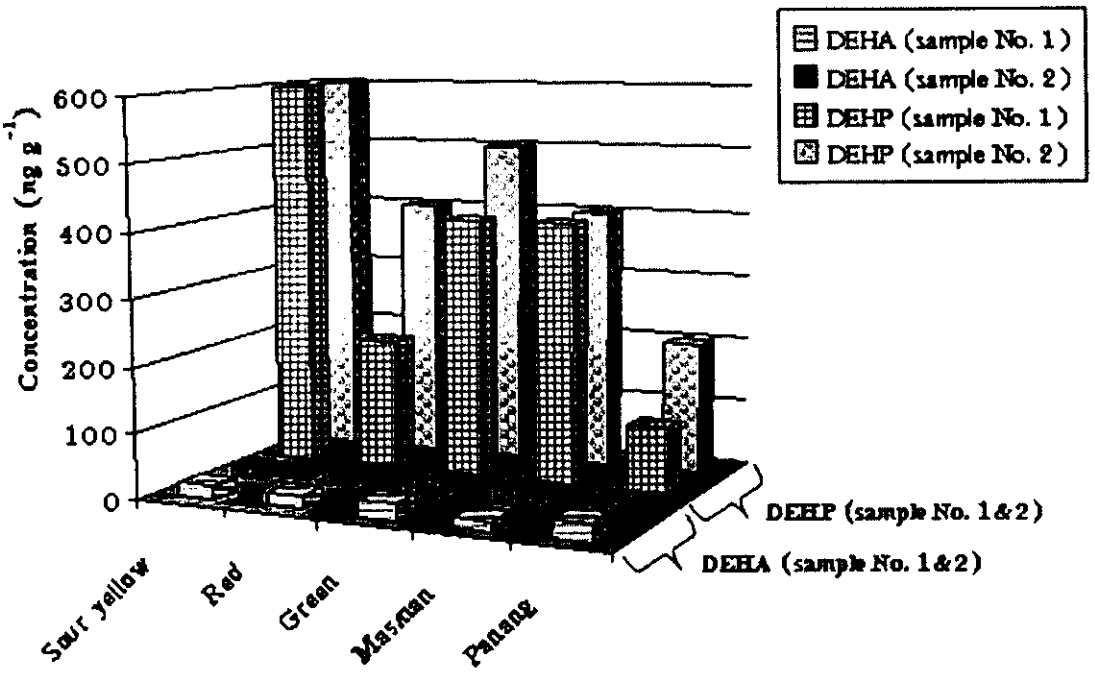

Figure 54 DEHP and DEHA levels in each type of curry paste sample (sample No. 1 and 2). Five replicates were done for each sample.

The concentrations of DEHP in all types of curry paste samples were extremely high when compared with DEHA. The major source of high DEHP may due to the used of plastic materials, such as thin laminar film inside the package that comes into close contact with the food. Moreover, the used of PVC gloves in the preparation of food is also the source of contamination because DEHP is a major plasticizer added to plastic production especially polyvinyl chloride (PVC). Disposable gloves have been widely used in packaging at factories to prevent microbial infection of foods since 1996. Disposable PVC gloves used in the preparation of foods were apparently the source of high DEHP concentrations. They contained 22 or 41% by weight of DEHP (Tsumura, 2001). In addition, It might have occurred through the ingredients or raw materials of the preparation of curry pastes because the vegetables that used as raw materials in curry paste can also accumulate phthalate from the environments (Gibson *et al.*, 2005; Horn *et al.*, 2004 and Sablayrolles *et al.*, 2005).

3.9 The regulations of phthalate and adipate esters contaminated in packaged food

The principal route of exposure of the general population to DEHP and DEHA is through food consumption. Food contact materials are all materials and articles intended to come into contact with packaged food, including packaging materials. It should be safe and should not transfer their components into the food in unacceptable quantities. The transfer of constituents from food contact materials into food is called migration. To ensure the protection of the health of the consumer and avoid any contamination of the packaged food, a Specific Migration Limit (SML) has been established for plastic materials which applies to individual authorised substances and is fixed on the basis of the toxicological evaluation of the substance. The SML is generally established according to the Acceptable Daily Intake (ADI) or the Tolerable Daily Intake (TDI) (EU legislation, 2004). These are used by the World Health Organization (WHO) and other national and international health authorities and institutes (ICH Q3B, 1997). TDI is the daily amount of chemical that could be consumed by humans on a per body weight basis without adverse effects on health (Drug Watch International, 2001).

The harmonisation at EU level of the legislation on food contact materials fulfils two essential goals: the protection of the health of the consumer and the removal of technical barriers to trade (EU legislation, 2004). Regulations and recommendations can be expressed as "not to exceed levels" are usually based on levels that affect animals (ATSDR, 2002). In Europe, the European Economic Community Scientific Committee for Food (EEC SCF) was requested to evaluate the hazards to human health arising from the migration into food of DEHA present in food contact materials. The committee concluded that a very small intake of DEHA from its use in food contact materials would not pose any carcinogenic hazard for man. It established a TDI of 0.3 mg kg^{-1} body weight (bw) for foetotoxicity in a teratogenicity study in rats (EU SCF, 2000). Although DEHP is unlikely to be as toxic to the testes of humans at the same level as in rats, the possibility of such testicular toxicity cannot be excluded at present. Therefore, in Europe, the EEC SCF has recommended a tolerable daily intake of 0.05 mg kg^{-1} bw day^{-1} for DEHP (SCF

Report 36th serie, 1997) and the Japanese Ministry of Health, Labor and Welfare (JMHLW) restricted the oral tolerable daily intake (TDI) value to 40 to 140 μg^{-1} kg^{-1} weight day^{-1} based on testicular and reproductive toxicities, respectively (Koizumi *et al.*, 2001; Haishima *et al.*, 2005).

Tables 55 and 56 show the one-time intake of phthalate and adipate esters from contaminated packaged food. The intake is given as a ratio to the body weight of the consumer. The calculated tolerable daily intake (TDI) of DEHP from curry paste samples were found in the range from 1.00×10^{-5} to 5.08×10^{-5} and 1.80×10^{-5} to 8.50×10^{-6} mg kg^{-1} bw day^{-1} when assumed that an adult (60 kg body weight) and a child (20 kg body weight) intake each curry paste 5 and 1 g per day, respectively. The intake is given as a ratio to the body weight of the consumer (Tsumura *et al.*, 2002). This intake, assumed that one packaged (50 g weight) of curry paste could be used to make curry for 10 adults and 50 childs based on normal Thai consumers who consumed moderate spices curry. For DEHA, the TDIs are in the range from 1.18×10^{-6} to 3.33×10^{-7} and 1.11×10^{-6} to 9.80×10^{-7} mg kg^{-1} bw day^{-1} for an adult and a child, respectively. The amount of DEHP and DEHA found in all curry paste samples in term of TDI does not exceed the level set by the EU tolerable daily intake (0.05 mg kg^{-1} bw day^{-1} for DEHP and 0.3 mg kg^{-1} bw day^{-1} for DEHA) and also less than TDI used in Japan (40 to 140 μg^{-1} kg^{-1} weight day^{-1}).

DEHP and DEHA detected in packaged food in this study were lower than the levels detected in previous study by Tsumura and coworker (2002), they found DEHP in a retort-pounched baby food sample at a level of 4.25 μg g^{-1} and found DEHA in butter at 2.78 μg g^{-1}. This survey showed a retort-pounched baby food sample to be contaminated by DEHP at the Japanese TDI level. The concentrations of DEHP and DEHA found in this work depend on several factors *i.e.*, type of curry paste (different ingredients), the length the sample is in contact with the plastic material, the lipid content in curry paste. It has been shown that DEHP and DEHA migration is highest when plasticized films come in direct contact with fatty food (Petersen and Breindahl, 1998; Tsumura *et al.*, 2002). The DEHP and DEHA contaminant in this work could either migrate from the plastic or the thin laminar film that is in close contact with the food, or from the food process that used plastic

that is in close contact with the food, or from the food process that used plastic products in manufacture. In addition DEHP and DEHA could also be contaminated from the vegetation surface such as chilli, galangal, lemon grass, mustard and turmeric, ground peanut, shallot and/or garlic that were used as raw materials for the curry paste. These results demonstrated that the plastic packaging is the major source of DEHP and DEHA contamination and their levels in packaged food are lower than the TDI.

Table 55 Intake of DEHP and DEHA from all curry paste samples when assumed that an adult (60 kg body weight) intakes each curry paste 5 g per day

Samples	Sample No.	Concentration (mg kg^{-1})		Intake per body weight (TDI) (mg kg^{-1} bw day^{-1})	
		DEHP	DEHA	DEHP	DEHA
Sour yellow curry paste	1	0.58	2.22×10^{-2}	4.83×10^{-5}	1.85×10^{-6}
	2	0.61	1.59×10^{-2}	5.08×10^{-5}	1.33×10^{-6}
Red curry paste	1	0.36	1.96×10^{-2}	3.16×10^{-5}	1.63×10^{-6}
	2	0.46	4.00×10^{-3}	3.83×10^{-5}	3.33×10^{-7}
Green curry paste	1	0.17	2.31×10^{-2}	1.42×10^{-5}	1.93×10^{-6}
	2	0.37	2.61×10^{-2}	3.08×10^{-5}	2.18×10^{-6}
Masman curry paste	1	0.37	1.41×10^{-2}	3.08×10^{-5}	1.18×10^{-6}
	2	0.38	2.35×10^{-2}	3.17×10^{-5}	1.96×10^{-6}
Panang curry paste	1	0.12	2.64×10^{-2}	1.00×10^{-5}	2.20×10^{-6}
	2	0.15	2.01×10^{-2}	1.25×10^{-5}	1.68×10^{-6}

Table 56 Intake of DEHP and DEHA from all curry paste samples when assumed that a child (20 kg body weight) intakes each curry paste 1 g per day

Samples	Sample No.	Concentration ($mg\ kg^{-1}$)		Intake per body weight (TDI) ($mg\ kg^{-1}\ bw\ day^{-1}$)	
		DEHP	DEHA	DEHP	DEHA
Sour yellow curry paste	1	0.58	2.22×10^{-2}	2.90×10^{-5}	1.11×10^{-6}
	2	0.61	1.59×10^{-2}	3.05×10^{-5}	7.95×10^{-7}
Red curry paste	1	0.36	1.96×10^{-2}	1.80×10^{-5}	9.80×10^{-7}
	2	0.46	4.00×10^{-3}	2.30×10^{-5}	2.00×10^{-7}
Green curry paste	1	0.17	2.31×10^{-2}	8.50×10^{-6}	1.16×10^{-6}
	2	0.37	2.61×10^{-2}	1.85×10^{-5}	1.31×10^{-6}
Masman curry paste	1	0.37	1.41×10^{-2}	1.85×10^{-5}	7.05×10^{-7}
	2	0.38	2.35×10^{-2}	1.90×10^{-5}	1.18×10^{-6}
Panang curry paste	1	0.12	2.64×10^{-2}	6.00×10^{-6}	1.32×10^{-6}
	2	0.15	2.01×10^{-2}	7.50×10^{-6}	1.01×10^{-6}