Chapter 1

Introduction

1.1 Introduction

The widespread use of plastics makes it almost impossible to prevent human from coming into contact with it, whether these people are workers in a production environment or consumers. Plastics appear to be a potential source of chemicals released into the environment, which may have a variety of effects on human health. Approximately 70 to 80% of food is packaged in various polymeric/plastic materials (Sheftel, 2000) and the principal hazardous factor associated with the use of plastics remains the possible contamination of food.

Plastics are manufactured by polymerization or polycondensation of one or more monomers and other starting substances. Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) (Sheftel, 2000) are widely used as basic polymers. The addition of additives to these polymers can alter the polymer properties or prolong the polymer life (Cano *et al.*, 2002). Some examples of additives are antioxidants, ultraviolet (UV) light adsorbers and plasticizers (Vandenburg *et al.*, 1997). Additives are added either during processing or fabrication of polymeric packaging. Plasticizers are the most common additives which are added to a material to improve its processability, flexibility and stretchability. They intersperse around the polymer molecules and prevent them from bonding to each other so tightly that they form a rigid substance. The major chemical types of plasticizers are polyester plasticizers, phthalate, adipate, trimellitate, phosphate, sulfonate, citrate, sebacate and azelate esters (The European Council for Plasticizers and Intermediates: ECPI, 2001). Among all plasticizers, phthalates and adipate esters are the most frequently used (Sheftel, 2000).

Phthalate esters are used in a wide range of products in homes, offices, hospitals and businesses for over 50 years. Among all phthalates, di (2-ethylhexyl)

phthalate (DEHP) is the most widely used in a variety of products, including medical devices, cabling, flooring and food packaging. It is present in many plastics, especially vinyl materials, which may contain up to 40% (ATSDR, 2002).

For adipates ester, the most commonly used is di (2-ethylhexyl) adipate (DEHA), which is used widely in food wrapping applications such as cling films (Risk & Policy Analysts Limited (UK), 2000). Plastic films and containers with DEHA as the plasticizer (up to 24 percent by weight of the polymer) are often used for storing and protecting food (FDA, 2000).

Since plasticizers are not chemically but only physically bound to the polymer chains (Staples *et al.*, 1997 and Ma *et al.*, 2003), they may leach into food and beverages from the plastic containers (Cray, 1998; Stringer *et al.*, 2000 and Li *et al.*, 2004). Therefore, the largest source of general population exposure to DEHP and DEHA is through food or dietary consumption (Petersen and Breindahl, 1998; Koch *et al.*, 2005), followed by indoor air (Kavlock *et al.*, 2002). Dietary exposures of DEHP and DEHA result both from accumulating in certain foods and leaching of them during processing, packaging, and storing (Kavlock *et al.*, 2002).

Phthalate and adipate esters are suspected of possessing endocrine disrupting effects (Harris *et al.*, 1997 and Hashimoto *et al.*, 2003) and regulations governing the use of these compounds in food contact material vary from country to country. In Europe, the European Economic Community Scientific Committee for Food (EEC SCF) was established a tolerable daily intake (TDI) of 0.05 and 0.3 mg kg^{-1} body weight day^{-1} for DEHP and DEHA. In Japan and Canada, the TDI level was set at 40-140 μg^{-1} kg^{-1} weight day^{-1} for DEHP.

In Thailand, the government wants to export Thai food internationally. The products would include prepared food, fresh and frozen items that include frozen seafood, chicken and pork; shrimp paste; seasonings like fish sauce and dried chilies; tinned coconut cream; dried medicinal herbs and curry pastes (Bangkok Post's newspaper, 2003). Thai curry paste, the foundation for flavouring Thai curries is the popular one and is mostly in plastic package. The analysis of DEHP and DEHA is, therefore, important.

In general food has a complex matrix sample, therefore, proper sample preparation procedures are necessary to achieve optimum analytical results. Conventional extraction techniques like liquid-liquid extraction (LLE) or solvent extraction are widely used for the extraction of phthalate and adipate esters in food (Simonean and Hannaert, 1999; Petersen and Breindahl, 2000; Tsumura *et al.*, 2002). These techniques have many disadvantages such as laborious, time consuming and require relatively large quantities of organic solvent which are carcinogenic and hazardous to the environment (Theodoridis *et al.*, 2000). These limitation can be reduced by using other extraction technique such as ultrasonic extraction, which is fast in comparison with the traditional methods, because of the contact surface area between solid and liquid phase is much greater, due to particle disruption taking place (Filgueiras *et al.*, 2000). In addition, the matrix of curry paste would be very different from other food samples *i.e.*, meat, retorted-pounched baby food, butter, margarine, milk, infant formula *etc.*, which have been studied by many researchers (Yin and SU, 1996; Petersen and Naamansen, 1998; Tsumura, *et al.*, 2002). Therefore, a suitable sample preparation technique is necessary.

In this work, a sample preparation method was developed for the analysis of phthalate and adipate esters using an ultrasonic extraction followed by solid phase extraction (SPE) technique with relative small quantity of solvent. The analysis was by gas chromatography equipped with flame ionization detector (GC-FID).

1.2 Background

1.2.1 Chemical identification

Phthalates are the class of chemicals known as dialkyl or alkyl aryl esters of *o*-phthalic acid (benzene-1,2-dicarboxylic acid) (Risk & Policy Analysts Limited (UK), 2000). Phthalates, like most plasticizers, are organic esters produced through reaction of a carboxylic acid and an alcohol. Di (2-ethylhexyl) phthalate (DEHP) is a branched-chain dioctyl ester of phthalic acid as shown in Figure 1. It is produced by reacting 2-ethylhexanol with phthalic anhydride (Risk & Policy Analysts

Limited UK, 2000) as shown in Figure 2. The reaction is either conducted in the presence of an acid or metal catalyst or at a high temperature (Kavlock *et al.*, 2002). DEHP dissolves more easily in materials such as gasoline, paint removers, and oils, than it does in water (ATSDR, 2002). The determination of the solubility in water and octanol/water partition coefficient of phthalic acid esters is complicated, since these compounds easily form colloidal dispersions in water.

Di (2-ethylhexyl) adipate (DEHA) is not known to occur in nature. It is manufactured by the reaction of adipic acid and 2-ethylhexanol in the presence of an esterification catalyst, such as sulfuric acid or *p*-toluenesulfonic acid (U.S. EPA, 2003). DEHA is commonly blended with general purpose plasticizers such as DEHP in processing polyvinyl chloride and other polymers (Richard and Lewis, 1993). The chemical structure of DEHA is shown in Figure 3. The difference between a phthalate and an adipate is in the different chemical structure and in the different raw materials. The first is based on phthalic anhydride while the second is based on adipic acid (European Council for Plasticizers and Intermediates (ECPI), 2005). Some details of DEHP and DEHA are shown in Table 1.

Table 1 Chemical identifications of DEHP and DEHA

Characteristic	DEHP	DEHA
Synnonyms	Bis (2-ethylhexyl) 1, 2-benzenedicarboxylate; Bis (2-ethylhexyl) *ortho*-phthalate; Bis (2-ethylhexyl) phthalate; Bisoflex 81; Bisoflex; DOP; Corflex 400; Ethylhexyl phthalate; 2-Ethylhexyl phthalate; Diacizer DOP; Dioctyl phthalate; Di (isooctyl) phthalate; Di-sec-octyl; phthalate; Octyl phthalate; Phthalic acid, Bis (2-ethylhexyl) ester; Phthalic acid di (2-ethylhexyl) ester; Phthalic acid dioctyl ester; Sconamoll DOP; Sicol 150; Staflex DOP; Truflex DOP; Vinycizer 80; Velsicol DOA; Witcizer 312	Adipic acid, bis (2-ethylhexyl) adipate; Bis (2- ethylhexyl) ester; Bis (2-ethylhexyl) Hexanedioate; Diethylhexyl adipate; Diacizer DOA; Dioctyl adipate(DOA); Effomoll DA; Effomoll DOA; Ergoplast AdDO; Flexol A 26; Hatcol 2908; Jayflex DOA 2; K 3220; Kodaflex DOA; Lankroflex DOA; Hexanedioic acid; Hexanedioic acid, Dioctyl ester; Monoplex DOA; NSC 56775; Octyl adipate; Rucoflex; Plastomoll DOA; Plasthall DOA; Reomol DOA; SP 100; Sansocizer DOA; Sicol 250; Truflex DOA; USS 700; Vistone A 10; Vestinol OA; Wickenol 158; Witamol 320
CAS registry number	117-81-7	103-23-1
Molecular formula	$C_{24}H_{38}O_4$	$C_{22}H_{42}O_4$

Source: Barbalace, 2004; Kayoko *et al.*, 2002; Tsumura *et al.*, 2001 and U.S. EPA, 2005

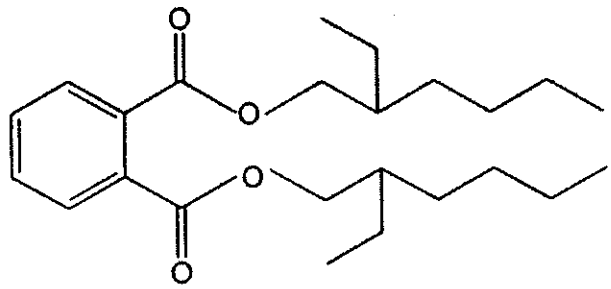

Figure 1 Chemical structure of di (2-ethylhexyl) phthalate (DEHP)

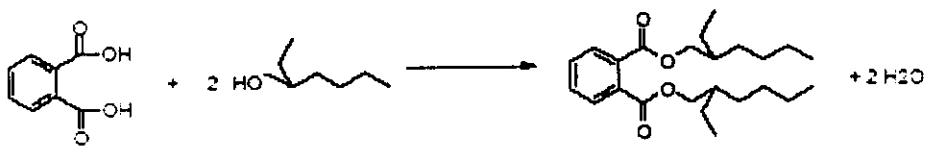

Figure 2 Production of di (2-ethylhexyl) phthalate
(Risk & Policy Analysts Limited (UK), 2000)

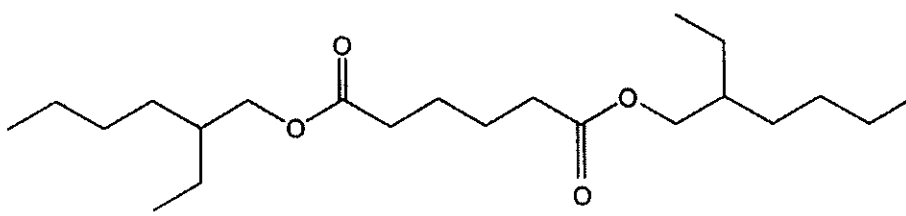

Figure 3 Chemical structure of di (2-ethylhexyl) adipate (DEHA)

1.2.2 Physical and chemical properties

DEHP is a colorless, oily liquid, with almost no odor (Canadian Environmental Protection Act, 1994), miscible with mineral oil and hexane (U.S. EPA, 1997). It does not evaporate easily, and little will be present in the air even near sources of production. DEHP dissolves more easily in materials such as gasoline, paint removers, and oils than it does in water (ATSDR, 2002). DEHA is a colorless or light-colored, combustible, oily liquid, with a slight aromatic odor. It is insoluble in water, glycerine and glycols, but soluble in alcohol, ether, acetone, acetic acid, and most organic solvents (Toxic Air Contaminant Identification, 1997). It is soluble in alcohol, ether, acetone, acetic acid, and most organic solvents. Its physical properties and uses are similar to phthalate esters such as DEHP (U.S. EPA, 2002). The physical and chemical properties of DEHP and DEHA are summarized in Table 2.

Table 2 Physical and chemical properties of DEHP and DEHA

Properties	DEHP	DEHA
Molecular weight (g mol^{-1})	390.62	370.84
Density (g mL^{-1})	0.986	0.922
Color	colorless, oily liquid	light colored liquid
Vapor pressure (mmHg)	6.2×10^{-8} at 25°C	8.5×10^{-7} at 25(°C)
Solubility in water	270 to 400 μg L^{-1} at 25 °C	0.78±0.16 mg L^{-1} at 22 °C
Melting point (°C)	-47 (°C)	-76 (°C)
Boiling point (°C)	384.9 (°C)	374.4 (°C)
Log K_{ow}*	7.5	6.3

Source: Canadian Environmental Protection Act (1994); WHO (1996); Tsumura *et al.* (2001); U.S. EPA (2002); Kayoko *et al.* (2002); Kavlock *et al.* (2002) and EPA (2005)

* Log K_{ow}: Partition coefficients octanol-water

1.3 Toxicity

Di (2-ethylhexyl) phthalate (DEHP) and di (2-ethylhexyl) adipate (DEHA), two chemicals that are used as primary plasticizers in flexible vinyl products, have been considered as possible health hazards. They are ubiquitous environmental contaminants and are therefore found at some concentration in virtually all people. High exposure being associated with toxicity. Phthalate and adipate esters are suspected to interfere with the endocrine system (Lee *et al.*, 2006),

"endocrine disrupting chemicals" that has the great concern all over the world (Murahashi *et al.*, 2003).

A number of chemicals in the environment are suspected to have endocrine-disrupting potential that might cause a decline in reproductivity of wildlife species. Endocrine disruptors are man-made synthetic chemicals and natural phytoestrogens (naturally occurring plant or fungal metabolite-derived estrogen) that act on the endocrine systems of humans and animals by mimicking, blocking or interfering in some manner with the natural instructions of hormones to cells. The disruption can take place as an inappropriate quantity or timing of a response to a stimulus, the blocking of hormonal effects in parts of the body normally sensitive to it, and the stimulation or inhibition of the endocrine system that could produce an inappropriate quantity of hormones too much, too little or none at all. Any combination of these interferences on the endocrine system can affect physical development, sex, reproduction, brain development, behavior, temperature regulation and more. Many of the observed endocrine-modulating and reproductive effects have been related to the phthalate and adipate esters, which are important industrial chemicals used as plasticizers in several plastic formulations. Comparing to the phthalates, less information is available on the toxicological profile of DEHA. It appears that DEHA has a toxicity profile similar to that of DEHP but with lower potency (Risk & Policy Analysts Limited (UK), 2000).

1.3.1 Di (2-ethylhexyl) phthalate (DEHP)

DEHP has been extensively studied due to its toxicity. Humans are exposed to DEHP via oral, inhalation, dermal and intravenous routes (Jarfelt *et al.*, 2005). Most of the toxicological studies were performed on rats, mice and other rodents. These animal species seem to be more sensitive to toxic effects of phthalates than humans. The critical organs are liver, kidney and testis (Heise and Litz, 2004). In recent years, some research articles have appeared discussing the impact of phthalate esters on wild animals and human. They suggested that increasing exposure to phthalate esters might be partially responsible for the recent decline in the male ratio and the development of breast cancer in humans. Furthermore, it was found that they

might persist in human body tissues for longer periods than previously assumed (Li *et al.*, 2004).

DEHP has attracted much attention because this compound is suspected of possessing endocrine disrupting effects (Harris *et al.*, 1997 and Bay *et al.*, 2005). The United States Environmental Protection Agency (U.S. EPA) also characterizes the cancer hazard of DEHP as a B2 group (probable human carcinogen), based on studies conducted by the National Toxicology Program (NTP) in the early 1980s using F344 rats and B6C3F1 mice where liver cancer was identified in both species (NTP, 1982 and Doull *et al.*, 1999). (Fisher (F) 344 rat and B6C3F1 mice are the specific strains of animals most frequently used by the NTP).

DEHP is animal carcinogen and also classified as reproductive toxicity and may cause small testicles and malformations in rodent studies. The extent of their toxicities and applicability to humans remains incompletely characterized and controversial. DEHP has received considerable attention recently because of specific concerns about pediatric exposures. Like all phthalates, DEHP is ubiquitous contaminants in indoor air, soils, sediments and food (Shea *et al.*, 2005). It exhibits low toxicity from acute (short-term) and chronic (long-term) exposures. Acute exposure to large oral doses of DEHP can cause gastrointestinal distress in humans. No information is available on the chronic, reproductive, developmental, or carcinogenic effects of DEHP in humans.

An enormous number of studies dealing with toxicological aspects of DEHP in animals have been published. A phenomenon that has attracted particular attention is referred to as "peroxisome proliferation" and is observed in animal experiments, most notably in mice and rats. This phenomenon, which is the strongest in the liver, is characterized by an increased number and size of peroxisomes (cell organelles involved in the metabolism of certain fatty acids), by induction of enzyme systems (*e.g.*, peroxisomal β-oxidation, carnitinacetyl- transferase, cytochrom P450 4A) and hepatomegaly (liver enlargement) due to hypertrophy (increased cell size) and hyperplasia (increased cell number). Peroxisomal induction has been associated with the formation of liver tumors although the mechanisms involved have not yet been fully elucidated. Mice and rats are the most sensitive species as far as peroxisome proliferation and tumor induction by peroxisome proliferators is

concerned whereas primates seem to be by far less responsive or non responsive. Consequently the relevance of this type of tumor formation to humans has been questioned. DEHP is not considered as genotoxic substance because it does not bind covalently to DNA (Jackh *et al.*, 1984) but it appears to be a tumor promoter. However, peroxisome proliferation induced tumorigenesis is not the only relevant toxicological endpoint. Several studies have shown that DEHP is embryotoxic and teratogenic in rodents. In addition, adverse effects on fertility on the male and female reproductive system including testicular atrophy (Kang *et al.*, 2006), reduction in sperm motility and concentration, increase in the number of abnormal sperm as well as histopathological damages have been observed (Fiala *et al.*, 2000). It is also reported that DEHP-treated male rats showed decrease in circulating testosterone levels and increase in serum estradiol levels. In addition, DEHP increased the frequency of proliferating MCF-7 human breast cancer cells in a dose-dependent manner, although it showed no estrogenic activity in recombinant yeast screen assays (Harris *et al.*, 1997). This toxicity of DEHP has been mechanistically related to peroxisome proliferators, hormonal dysregulation, and free radical generation. Estrogen is also metabolically activated to catechol metabolites and further to semiquinone or quinone, leading to free radical generation, which might be an important mechanism for estrogen toxicity (Lee, 2001).

DEHP also causes skeletal, cardiovascular, and eye abnormalities, neural tube defects, intrauterine death, increased postnatal death, and decreased intrauterine and postnatal growth in rodent pups whose dams received DEHP in feed or by gavage during pregnancy. A "lowest observable adverse effect level" (LOAEL) is observed with fetal toxicity occurring at the same dose or a lower dose than that causing mild maternal toxicity. Thus, fetal toxicity could occur without evidence of maternal toxicity after oral exposure. The most sensitive system is the reproductive tract of immature males. Pathologic changes in the testes and decreased sperm numbers are consistent effects across studies. Changes in weight of the testes, and atrophy of the seminiferous tubules have been observed in rodent pups exposed to DEHP in utero via dietary exposure of dams (LOAEL, 38-141 mg kg^{-1} per day; NOAEL, 3.7-14 mg kg^{-1} per day) (Shea *et al.*, 2005).

1.3.2 Di (2-ethylhexyl) adipate (DEHA)

Like DEHP, DEHA is also associated with endocrine system effects both in animals and humans and *in vitro* (Hashimoto *et al.*, 2003). It is a widely used plasticizer in PVC films employed in food wrapping material. It is a high priority for revaluation because of reproductive and/or developmental concerns (U.S. EPA, 2003). DEHA appears to be readily absorbed when given orally to rats and mice. It is widely distributed in the body; the highest levels have been reported in adipose tissue, liver, and kidney (WHO, 2004). DEHA is initially hydrolysed to mono (2-ethylhexyl) adipate (MEHA), adipic acid and 2-ethylhexanol (Dalgaard *et al.*, 2003), which are excreted as such or further oxidized to several different compounds before being eliminated in the expired air, urine, and faeces of experimental animals. Major metabolites of DEHA are MEHA and its glucuronide, the glucuronide of 2-ethylhexanoic acid, and adipic acid. Single oral doses of DEHA seem to be completely excreted by rats, mice, and monkeys in 48 hours (WHO, 1996).

DEHA has been shown to be a liver carcinogen in mice of both sexes, but no treatment-related tumors could be found in rats (IARC, 2000). Developmental studies have shown that DEHA decreases pup weight at high dose levels. To date, no investigations of possible anti-androgenic effects have been reported even though there are similarities in chemical structure and metabolism of DEHP and DEHA. The critical period of susceptibility to endocrine disrupters including the phthalates appears to be during sex differentiation and development of the reproductive system. Endocrine changes during development may disturb adult reproductive performance (Dalgaard *et al.*, 2003). Acute (short-term) exposure in mouse and rat toxicity studies have demonstrated that high dietary levels of DEHA (6000 mg kg^{-1}) induce liver toxicity, including increased liver weights, histopathological liver changes, and proliferation of liver peroxisomes, accompanied by increased activities of catalase and of enzymes involved in the oxidation of fatty acids as well as hypolipidaemia. Chronic (long-term) exposure studied in rat and mice at dietary levels of 12000 or 25000 mg kg^{-1}, no dose-related effect on longevity was seen. A dose-related depression of growth rate was observed in mice. Except in the liver, where tumors developed, no histopathological changes were observed in the

mouse (WHO, 1996). DEHA has very low acute toxicity, the following LD_{50} values have been reported: Rat (oral) 7392-45000 mg kg^{-1} body weight (bw); mouse (oral) 15000-24600 mg kg^{-1} bw; rabbit (dermal) 8410-15100 mg kg^{-1} bw. The symptoms of intoxication in the rat following oral administration were coordination disorders (European commission, 1999).

The carcinogenic potential of DEHA has also been investigated, while there are no human data, there are two animal cancer studies on the compound conducted by NTP (1982). The report from WHO (1996) show that DEHA was not carcinogenic for F344 rats but DEHA was carcinogenic for female $B6C3F_1$ mice, causing increased incidences of hepatocellular carcinomas, and was probably carcinogenic for male $B6C3F_1$, causing hepatocellular adenomas". DEHA belongs to a group of chemicals called peroxisome proliferators, which are characterized by their ability to induce hepatic peroxisome proliferation, especially in rodents. Peroxisome proliferation is visible microscopically as a massive increase in the number of peroxisomes, small membranous organelles that contain various oxidative enzymes. DEHA-induced peroxisomal proliferation with accompanying biochemical events was found to be a dosedependent phenomenon (WHO, 1999). Many synthetic chemicals are peroxisome proliferators including plasticizers (*e.g.*, DEHP and dibutyl phthalate; DBP). Some of the peroxisome proliferators have been shown to cause liver tumors as well as pancreatic tumors, testicular tumors and tumors of the hematopoietic system in rats and mice. The evidence of cancer in DEHA-treated rodents is limited. Inter-species comparisons with other peroxisome proliferators, along with the role of peroxisome proliferator-activated receptors in this response, indicate that humans may be less sensitive than rodents to induction of peroxisome proliferation and hepatocellular proliferation by DEHA (IARC, 2000a, OEHHA, 2001).

The DEHA carcinogenicity data have been reviewed by IARC (2000) and U.S. EPA (1999). Based on the genotoxicity and carcinogenicity data available, IARC (2000) determined that DEHA was not classifiable as to its carcinogenicity to humans (Group 3). U.S. EPA (1999) classified DEHA as a Group C (possible human carcinogen) and developed an oral slope factor of 1.2×10^{-3} (mg kg^{-1} day^{-1}) for the compound (U.S. EPA, 2002). For toxicological effects in humans, no studies were found in the available literature on the effects of oral ingestion of DEHA

in humans (U.S. EPA, 2002). By combining DEHA with DEHP, DEHP seems to advance in antiandrogenic effects while no modulating effect of DEHA on the antiandrogenic effects of DEHP was observed. Because of the antiandrogenic effects of DEHP, DEHA is being evaluated as a potential substitute for DEHP (Jarfelt *et al.*, 2005).

1.4 Literature reviews

Phthalate and adipate esters have received attention as potential environmental contamination due to its adverse health effects and endocrine disrupting properties. The Canadian Environmental Protection Act (CEPA) classified the commonly occurring phthalates as priority pollutants that may be harmful to the environment or constitute a danger to human health (CEPA, 1993). Human beings are continuously exposed to many products containing phthalate and adipate esters (Basheer and Lee, 2002). The exposure to DEHP may arise from toys and childcare articles, building materials and home furnishing, medical devices and food contact materials (Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), 2004).

1.4.1 Occurrence in food

General population may come in contact with DEHP and DEHA by several sources of exposure, but the main exposure is dietary or food consumption (Latini *et al.*, 2004). Because of the potential health impact on human, several studies have been performed to assess the presence of DEHP and DEHA in food. PVC film is still widely used as a food wrapping material due to its flexibility, transparency and low water permeability. In the form of thin film, also known as "cling film" it is used for retail packaging of food, such as red meat, poultry, cheese, fruit and vegetables (Zygoura *et al.*, 2005). The migration of DEHA from PVC film has been investigated in a variety of foods. Page and Lacroix (1995) reported a Canadian study that analyzed DEHA in 98 food samples. They evaluated DEHA in food-contacting film and as a migrant in store-wrapped meat, poultry, fish, cheese and ready to eat food.

The investigators reported that on a whole food basis, DEHA at levels up to 9.5, 14, 220 and 310 μg g^{-1} was found in chicken breast, regular ground beef, smoked salmon fillet, and cheese, respectively. DEHA in non-contacting or "core" samples obtained from several of the meat and chicken samples was below the detection limit (< 0.4 μg g^{-1}). The DEHA levels found in the interior core of the cheese samples were about 1-7% of the levels found in the whole food. These results demonstrated that the retail wrap is the source of DEHA contamination.

The level of contamination also depends on the type of food. Petersen and Breindahl (1998) showed that DEHA migration was highest when plasticized PVC films come in direct contact with fatty food because lipophilic characteristics of DEHA may operate as the driving force accelerating the migration phenomenon in substance (Kim *et al.*, 2003). Different types of cheese samples were wrapped with food-grade PVC film containing 28.3% DEHA plasticizer. The effect of cheese rind on migration of DEHA was studied in two brands of cheese. The DEHA migration after 240 hours into the 1 mm beneath the surface of cheese was found with the highest concentration at 22.4 mg kg^{-1} (Goulas *et al.*, 2000).

In other works, diet samples were tested. Petersen and Breindahl (2000) analyzed 29 adult diet samples, 11 baby food samples and 11 samples of infant formula. They found DEHA in 18 adult diet samples, none of the baby food samples, and 2 of the infant formula samples. The average DEHA concentrations in the adult diet and infant formula samples were 140 and 35 μg kg^{-1}, respectively. In Japan, DEHA in diet samples was monitored to estimate daily intake. Daily diet samples consisting of breakfast, lunch and supper were obtained from hospitals over a period of one week and analyzed for DEHA and eleven phthalate esters. Daily intake of DEHA was found to vary from hospital to hospital and from day to day. The estimated intake level ranged from non-detect to 429 μg day^{-1}, with an average of 86 μg day^{-1} (Tsumura *et al.*, 2001).

For phthalate esters, a surveillance work in Canadian dairy products and margarine has shown that the levels of DEHP and butylbenzyl phthalate (BBP) were up to 11.9 and 47.8 mg kg^{-1}, respectively (Page and Lacroix, 1992).

Although government of canada does not have permissible level of phthalates in milk or dairy products, it has recommended a tolerable daily intake (TDI) of 44 μg kg^{-1} (body weight) day^{-1} for DEHP, where the exposure of Canadians to DEHP from all sources was estimated at 5-19 μg kg^{-1} (body weight) day^{-1} depending on age (Priority Substances Assessment Report, 1994). Feng *et al.* (2005) determined phthalate esters in raw cow milk from dairy farms. The level of DEHP was found much higher in samples collected using PVC tubing (215.36 ng g^{-1}) than one without (16.04 ng g^{-1}), indicating potential leaching of DEHP from PVC tubing into raw cow milk.

1.4.2 Occurrence in food packaging

Phthalates in packaged food depends on many factors including the concentration of phthalates in the packaging material or printing ink, the storage period, the storage temperature, the fat content in the food and the contact area (Balafas *et al.*, 1999). The presence of phthalates in packaging materials and their migration into packaged foods have been confirmed by a number of authors (Page and Lacroix, 1995; Aurela *et al.*, 1999 and Chen *et al.*, 2004). The predominant plasticizers in bread packaging were DEHA, followed by DEHP and DBP. Low levels of DEHP (0.065 μg g^{-1}, average in beverages and 0.29 μg g^{-1}, average in food) associated with the use of DEHP-plasticized cap or lid seals, were found in a variety of glass-packaged foods (food in glass containers such as butter and margarine); dibyl phthalate (DBP), butylbenzyl phthalate (BBP) and DEHP were found in butter and margarine as migrants from the aluminium foil-paper laminates; and diethyl phthalate (DEP) in pies at 1.8 μg g^{-1} (average) as a migrant from the pie carton windows. In most cases, plasticizers detected in the food were also found in the associated packaging (Page and Lacroix, 1995). The amount of phthalate and adipate esters in Australian packaging materials in 14 bread packaging samples were studied by Balafas *et al.* (1999), the results showed that they contained at least two of the targeted packaging additives. The material analysed during the 12 month period was all printed polyethylene (PPE). Total phthalate concentrations ranged from 27 to 295 μg g^{-1}. dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-*n*-octyl phthalate

(DOP) were not present in any samples. BBP was present in only three samples in identical trace amounts (5 $\mu g\ g^{-1}$).

1.4.3 Occurrence in childcare article and medical device

Dialkyl phthalates have been used as plasticizers in many household products made from polyvinyl chloride (PVC), including children's products such as soft plastic teethers, rattles, and toys. Because of plasticizers are not tightly bound to PVC, they may be released when children place PVC products in their mouths by sucking and biting (Babich *et al.*, 2004). Health Canada stated that a year-long review found that children weighing under 8.2 kg, who suck on the products for more than 3 hours a day, run the risk of liver enlargement or kidney scarring (Kondro, 1998). This is an issue of serious health concern, which gave rise to numerous discussions about the risk to children. The EU adopted measures prohibiting the placing on the market of toys and childcare articles intended to be placed in the mouth by children under 3 years of age made of soft PVC containing one or more of the substances diisononyl phthalate (DINP), BBP, DBP, DOP and DEHP.

Plastic materials are also widely used in medical items, such as solution containers, associated closures, delivery sets, transfer tubing, and devices. The physiochemical nature and composition of these materials provide medical products with their necessary, desirable performance characteristics. While an important performance characteristic of plastics used in medical applications is chemical inertness, interactions between a plastic material and a contacted pharmaceutical product are well documented (Sarbach *et al.*, 1996; Jenke *et al.*, 2006). Such interactions include leaching of the release of plastic material components to the product since it can potentially impact product safety (Jenke *et al.*, 2005). DEHP is the most widely used plasticizer for PVC medical devices (Adams, 2001). The functional characteristics and processability of the material makes it very suitable for the construction of a wide variety of these devices, some of which are crucial to the delivery of care to critically or chronically ill patients. It is recognised that DEHP is able to diffuse through PVC and may leach out into its environment,

including the environment of human body, should a DEHP-PVC article be in contact with that body. As a result of this leaching process, patients may be exposed to DEHP. The extent of this exposure will vary, depending on device, treatment and individual variables. It is recognised that there may be multiple sources of exposure, including not only the specific medical device in question but also on the presence of PVC containing products in the general hospital, clinic or home environment, and on general environmental factors, including the presence of DEHP in water, food and the air (European Commission, 2002).

Another study shows that babies receiving intensive therapy with PVC medical devices were exposed to the phthalate at levels on average 25 times higher than the general population and up to 50 times higher for the most exposed (Environmental, Health and Safety Issues, 2005). It is recognised that a lack of data does not lead to a conclusion that DEHP is without adverse effects. Specifically it is agreed that in critically ill neonates, who constitute an inherently high-risk group of patients, the lack of evidence of causation between DEHP-PVC and any disease or adverse effect does not mean that there are no risks. On the basis of the evidence presented in this report, no tolerable intake value for DEHP in medical devices can be recommended (European Commission, 2002). The analysis of plasticizers like DEHP from the inner surface of the blood bags has been studied by Chen *et al.* (2004). The analysis showed an increase in detected DEHP after 10 days, which might be due to accumulation. However, the observed decrease in concentration after 15 days might be due to the enzymatic degradation of DEHP by the enzymes present in the blood, physically adsorbed on the inner surface of the blood bags.

1.4.4 Occurrence in environment

The phthalates enter to the environment during production, manufacturing (minor pathway) and by leaching, migration and volatilisation (major pathway) during use and after disposal of the products (Heise and Litz, 2004). It has been demonstrated that plasticizers tend to leach from solid polymer matrices into the environment (Fromme *et al.*, 2002). Numerous studies have confirmed the presence of plasticizers in air, soil and water samples to such an extent that they are now

described as being ubiquitous in the environment (Fromme *et al.*, 2002). The phthalates that found ubiquitously are primarily DEHP, and in much lower concentrations DBP and BBzP (Heise and Litz, 2004). DEHP has been found everywhere in the environment, and is universally considered to be an ubiquitous environmental contaminant (Latini *et al.*, 2004). It is released to the environment through volatilization and leaching from plastics and other sources. Its widespread usage coupled with its persistence in the environment result in its ubiquitous presence in the environment and in biota including humans (Feng *et al.*, 2005)

Sewage treatment plant effluents contain very low DEHP concentrations compared to raw sewage because DEHP readily adsorbs to the solid particles of the sludge. Effluents contained only a few μg L^{-1} of DEHP, *i.e.*, concentrations similar to those found in river water (Marttinen, 2003). DEHA was also found at μg L^{-1} levels in two out of five samples of finished water from a waste treatment plant in the USA. A survey of 23 major rivers and lakes in the USA showed that 7% of the samples contained DEHA at levels ranging from 0.25 to 1.0 μg L^{-1} (WHO, 2004). Beside soil and water, the presence of phthalate esters in atmosphere was also investigated. The area studied was in the urban area Paris (France). Total atmospheric levels (ng m^{-3}) were as follows: DMP, 0.5; DEP, 10.7; DnBP, 22.2; BBP, 4.6; and DEHP, 18.9 ng m^{-3}, showing a predominance of DnBP and next, DEHP. They are mainly present in the vapour phase, from 93.8% to 64.9% respectively (Teil *et al.*, 2006).

1.4.5 Analysis method

The determination of phthalate and adipate esters in food is carried out in two steps: (i) sample treatment, in which analytes must be separated from other interference components that are present in sample matrix, and (ii) measurement, in which analytes are measured using a suitable technique (Cano *et al.*, 2002). Because of the widespread use of phthalate and adipate esters, numerous applications require these chemicals to be controlled. Generally, the analytical method chosen involves the use of a separation technique with a suitable detector for identification and quantification of phthalates and adipate esters in the samples. The

analytical techniques applied to the determination of these compounds, mainly Gas chromatography (GC) and high-performance liquid chromatography (HPLC), are well established (Cano *et al.*, 2002).

Gas chromatography is the most common analytical method for detecting and measuring DEHP and DEHA in food (Castle *et al.*, 1988; Petersen and Breindahl, 2000; Tsumura *et al.*, 2002; Tsumura *et al.*, 2000; Fankhauser-Noti and Grob, 2005). High performance liquid chromatography (HPLC) might also be employed (Dine *et al.*, 1998; Castillo and Barceló, 2001 and Kambia *et al.*, 2002).

Most GC methods involve the use of columns based on dimethylpolysiloxane and/or phenyl-methylpolysiloxane non-polar, bonded and cross-linked stationary phases (Gómez-Hens and Aguilar-Caballos, 2003). The separation of phthalate and adipate esters by GC requires a relatively wide range of temperature programming. Detectors used to identify DEHP and DEHA include the electron capture detector (ECD) (EPA method 8061A, 1996) and the flame ionization detector (FID) (Marín *et al.*, 1996; Sverdrup *et al.*, 2000; Song *et al.*, 2000 and Wang *et al.*, 2000). When unequivocal identification is required, a mass spectrometer (MS) coupled to the GC column might be employed (ATSDR, 2002). Reverse-phase HPLC using C_{18} columns has been used to a lesser extent than GC for the separation of phthalates (Dine *et al.*, 1996; Kelly and M. Larroque, 1999 and Petrovic and D. Barceló, 2000; Castillo and D. Barceló, 2001). Both isocratic and gradient elution were described for this purpose. The detection in these methods has usually used UV detector (Dine *et al.*, 1996; Kelly and M. Larroque, 1999; Jara *et al.*, 2000 and Kambia *et al.*, 2001) although, in some cases, a MS detector was used (Petrovic and D. Barceló, 2000 and Castillo and D. Barceló, 2001). Reproducible gradients with short equilibration time were obtained in a normal phase HPLC method (Meyer, 1997), using a silica-packed column, for the separation of a test mixture containing 10 compounds of low to moderate polarity, including DEHP. Generally, GC methods obtain better LODs than HPLC methods, although they depend on the pre-treatment step, the instrumental conditions and the sample matrix in which they are obtained. When compare between GC and HPLC, HPLC is used less, one reason being the much better chromatographic resolution of single compounds with capillary columns compared to HPLC. Another reason is the common use of mass selective detectors in

GC while these detectors are not used as frequently in HPLC (Heise and Litz, 2004). A summary of the application of GC to analyse phthalate and adipate esters is as shown in Table 3.

Table 3 Application of GC to the determination of phthalates and adipate esters

Sample	Pre-treatment	Detection	Reference
Ground water, leachate, soil, sludge and sediment	Solvent extraction, Clean up with florisil and alumina cartridge, gel permeation chromatography (GPC)	ECD	EPA method 8061A, 1996
Landfill leachates	Solvent extraction (hexane)	MS	Yasuhara *et al.*, 1997
Fresh meat	Sonication, Clean up with GPC	FID	Petersen and Naamansen, 1998
Food simulants	Liquid-liquid extraction, Sonication	FID	Simonean and Hannaert, 1999
Food-packaging materials	Soxhlet extraction (chloroform/methanol)	MS	Balafas *et al.*, 1999
Medical products	Thermodesorption	MS	Wahl *et al.*, 1999
Food	Solvent extraction (ethylacetate/cyclohexane)	MS	Petersen and Breindahl, 2000
Sewage sludge	Ultrasonic extraction with cyclohexane	MS	Tienpont *et al.*, 2001
Natural water	SPME	MS	Penalver *et al.*, 2001

1.4.6 Sample preparation

Food analysis is important for monitoring food additives and other toxic contaminants. Sample preparation, such as extraction, concentration and isolation of analytes, is a critical step in the overall processes of obtaining reliable and accurate data for many analytical methods (Kataoka *et al.*, 2000). Samples usually must be processed in order to isolate and concentrate organic analytes from the sample matrix and provide a suitable sample extract for instrumental analysis. Several sample preparation techniques have been developed for determining phthalate and adipate esters such as solvent extraction from solids, liquid liquid extraction (LLE) from solutions (Ke *et al.*, 2000), solid-phase extraction (SPE) (Davi *et al.*, 1999; Jara *et al.*, 2000 and Jonsson and Born, 2002), and solid-phase microextraction (SPME) (Fattore *et al.*, 1996; Peñalver *et al.*, 1999; Peñalver *et al.*, 2000; Peñalver *et al.*, 2001; Zeng *et al.*, 2001; Prokupkova *et al.*, 2002; Saito *et al.*, 2002 and Cai *et al.*, 2003). Among these reports, sample matrices, such as polyvinyl chloride (PVC) plastic products were the most common ones, since phthalates were most commonly used plasticizers in PVC-based products due to their compatibility and softening capability (Cano *et al.*, 2002). Other matrices were environmental samples, including water and soils (Castillo and Barceló, 1997 and Zeng *et al.*, 2004).

1.4.6.1 Liquid-liquid extraction (LLE)

Conventional liquid-liquid extraction (LLE) or solvent extraction is based on the partition of organic compounds between the aqueous sample phase and an immiscible organic solvent (Christian, 1994), which is non- or just slightly polar. Hexane and cyclohexane are frequently used for compounds with aliphatic properties, whereas dichloromethane and chloroform are popular solvents for non to medium-polar contaminants. LLE procedures employ a serial extraction of an aqueous sample with an organic solvent resulting in a relatively large volume of solvent that must be dried and concentrated to a few millilitres by rotary evaporation or nitrogen stream prior to analysis (Beney *et al.*, 2004). Advantages of this technique are the simplicity of the procedure and inexpensive equipment (mostly glassware).

Numerous methods have been developed for almost any analyte and most official bodies accept this technique as part of an official method. Disadvantages include contamination and loss of sample (by adsorption to the glassware) due to several sample handling steps. Large volumes of organic solvents (hydrocarbons, chlorinated solvents, *etc.*) which are often expensive, toxic, carcinogenic and hazardous to the environment (Theodoridis *et al.*, 2000). Solvents with high purity have to be used in trace analysis contributing to the high costs of the analyses. Time-consuming and with an increasing number of analytes this will cause a solvent waste problem (Wahl *et al.*, 1999). Usage in the field is not easy and methods can only be performed off-line.

Liquid-liquid extraction has been used for analysis of 9 phthalate esters in fresh water as markers of contamination sources by transferring 0.5 L water sample in to a separatory flask and extracting with dichloromethane. After filtration, it was evaporated to dryness and reconstituted with isooctane, phthalate esters were determined by GC-MS. The recoveries ranged from 50-105% and limits of detection of the analytical method was 0.006-0.012 $\mu g\ L^{-1}$ (Vitali *et al.*, 1997).

The extraction of solid sample is most commonly done using traditional liquid-solid extraction methods. The most common of these approaches is soxhlet extraction offering an even cheaper alternative. Soxhlet extraction was introduced by Baron von Soxhlet in the mid-nineteenth century. Extraction involving transfer of analytes into an organic solvent are not limited to liquid samples or solutions. In soxhlet extraction, the solid sample is tested, distilled solvent drips into the porous thimble, immersing the solid sample. When the thimble is full, solvent is siphoned back into the solvent reservoir and redistilled. Soxhlet extraction is generally used for semi- or nonvolatile analytes as volatiles may be lost through the condenser. In this technique, only one sample can be extracted per set of apparatus, but it is possible to operate with as many sets of apparatus as space in a fume cupboard allows. Another disadvantage is that soxhlet extraction is usually slow, often requiring several hours (Grob, 2004).

Solvent extraction has been widely used for the determination of phthalates in solid samples, such as food (Petersen and Breindahl, 2000; Tsumura, *et. al*, 2001; Summerfield and Cooper, 2001), food packaging materials (Aurela *et al.*, 1999; Balafas *et al.*, 1999 and Song *et al.*, 2000), PVC articles (Rastogi, 1998; Steiner

et al., 1998 and Fiala *et al.*, 2000) and sewage sludges (Petrovic and Barceló, 2000; Berset and Etter-Holzer, 2001 and Tienpont *et al.*, 2001). The analysis in food packaging materials has been studied by Balafas *et al.* (1999). They analysed phthalate esters and one adipate ester in 136 food packaging materials. All of the sample materials such as polyethylene (PPE), polystyrene (PS), polyethylene (PE), polyethylene terephthalate (PET) were in immediate contact with their food contents. The esters in the packaging materials were extracted into a 2:1 mixture of chloroform and methanol, and analysed by GC-MS. All of the materials examined were found to contain two or more of these compounds above a detection limit of 0.01 mg kg^{-1}.

Recent migration studies (Goulas *et al.*, 2000; Summerfield and Cooper, 2001 and Fankhauser-Noti and Grob, 2005) show interest in better understanding the transfer of phthalate from packaging materials to food, as this migration process is a well-known source of food contamination. The determination of plasticizers in food by GC-MS was describes by Lau and Wong (1996). Fatty and non-fatty foodstuff were extracted by solvent extraction with dichloromethane-cyclohexane (1:1, v/v) followed by the gel-permeation chromatographic clean up procedure. The recoveries of the plasticizers were in the range of 90-106%. The relative standard deviation (RSD) less than 10%. The major problem of using solvent in the sample preparation is that residues of phthalate esters, especially DEHP, are also found in solvents (Wahl *et al.*, 1999). Furthermore, fat in dairy product samples such as milk could be co-extracted and require extra steps to remove them prior to analyte by instrument analysis (Feng *et al.*, 2005).

1.4.6.2 Ultrasonic extraction

Ultrasonic extraction or sonication is an alternative to conventional extraction techniques such as liquid liquid and soxhlet extraction. This technique has the benefit of shortened extraction times (Leblanc, 2001) and also provides a more efficient contact between the solid and solvent (Li *et al.*, 2004). It is an inexpensive and simple extraction technique. Ultrasonic extraction uses mechanical energy in the form of a shearing action, which is produced by a low-frequency sound wave to agitate the sample that immersed in organic solvent

(Leblanc, 2001). The mechanical effects of ultrasound induce a greater penetration of solvent into samples and improve mass transfer. Therefore, an effective mass transfer is leading to the enhancement of extraction with ultrasonic power (Wang and L. Weller, 2006).

In ultrasonic extraction, the sample is placed in a suitable glass container and enough organic solvent added to cover the sample and placed in an ultrasonic bath. The sample is then sonicated using the sonic bath or probe. The efficiency of extraction depends on the polarity of the solvent, the homogeneity of the matrix and the ultrasonication time. The mixture of sample and organic solvent is separated by filtration and washing with the solvent (Hess *et al.*, 1995). Ultrasonic extraction is easy to use and does not require expensive instruments (Ahmed, 2003). After extraction, the solvent containing the analyte can separated by centrifugation and/or filtration and fresh solvent added. The whole process is repeated three times and all the solvent extracts combined (John R, 1998).

Petersen and Naamansen (1998) applied ultrasonic extraction technique for analysis of DEHA in fresh meat after packaging and storage with PVC films. until their use by date. The samples extracted in an Ultra-Turrax with acetone-pentane (1:1, v/v) as an extraction solvent. The combined extracts were then filtered through cotton wool, dried and evaporated until dryness. The fat was redissolved in ethylacetate: toluene (3:1, v/v) and 2 mL was injected onto a gel permeation chromatography (GPC) column packed with Biobeads S-X3. The eluate fraction containing DEHA was collected and injected into an GC-FID. DEHA recovery of spiked samples by this method was 106% with the coefficient of variation (CV) for 11 duplicate determinations of spiked samples was 14.9%. The limit of determination was 0.6 mg DEHA kg^{-1} fresh meat.

1.4.6.3 Solid Phase Extraction (SPE)

Solid Phase Extraction (SPE) or sometimes referred to as liquid-solid extraction technique has been developed to replace many traditional liquid-liquid extraction methods for the determination of organic analytes in aqueous samples. It has drastically changed the classical approaches of solvent extraction. This

process, widely used for trace analysis (Rouessac and Rouessac, 2000). Using SPE, multiple samples can be treated in parallel using relative small quantities of solvent (Buldini *et al.*, 2002; Dopico-García *et al.*, 2005 and Hercegov′ *et al.*, 2005).

In the analysis of phthalate and adipate esters, sample matrices, such as polyvinyl chloride (PVC) plastic products were the most common ones, since phthalates were most commonly used plasticizers in PVC-based products due to their compatibility and softening capability (Cano *et al.*, 2002). Shen (2005) studied the determination of eight phthalates, *i.e.*, di-ethyl phthalate (DEP), di-propyl phthalate (DPP), di-isobutyl phthalate (DIBP), di-butyl phthalate (DBP), benzyl butyl phthalate (BBP), di-cyclohexyl phthalate (DCHP), di-(2-ethylhexyl) phthalate (DEHP), di-octyl phthalate (DOP), in 25 kinds of plastic products for food use, including packaging bags, packaging film, containers, boxes for microwave oven use, sucking tubes, spoons, cups, plates, *etc.* by gas chromatography in combination with mass spectrometry detector (GC-MS). Determination of samples were performed after frozen in liquid nitrogen and sonication-assisted extraction with hexane, clean-up with C_{18} SPE and analyzed by GC-MS methods. These techniques are possible to detect phthalates at the level of 10 $\mu g\ kg^{-1}$. Overall recoveries were 82-106% with RSD values at 3.8-10.2%. The predominant phthalate detected in the studied samples was DEHP (Shen, 2005).

Other matrices, including water, soils (Castillo and Barceló, 1997) and food have been studied. Brossa *et al.* (2003) developed an automated on-line solid phase extraction (SPE) gas chromatography mass spectrometry method to determine a group of endocrine disruptors in water samples including DEHP and DEHA. The interface device used for connecting SPE with GC was a programmed-temperature vaporiser (PTV). The limits of detection of the method were between 0.001 and 0.036 $\mu g\ L^{-1}$ under full-scan acquisition mode. The experiments showed that there were no carry over effects in the procedure. The RSD, varied from 1 to 8% for repeatability, except for DEHP (20%), and from 3 to 25% for reproducibility. The recoveries for DEHP and DEHA are in the range 72-98%. In general, these recoveries are better than those obtained in the earlier study by Brossa *et al.* (2002) that are in the range 58-83%.

In food analysis, Tsumura *et al.* (2000) reported the determination of 11 phthalate esters (diethyl, dipropyl, dibutyl, dipentyl, dihexyl, butylbenzyl, dicyclohexyl, di (2-ethylhexyl), dioctyl, diisooctyl, diisononyl) and di (2-ethylhexyl) adipate in oneweek duplicate diet samples obtained from hospitals. Homogenized samples of composite meals were extracted with acetonitrile, lipids were removed by extraction into *n*-hexane and the acetonitrile layer was cleaned using Florisil^R and Bondesil PSA^R dual layer column. Phthalates were determined by GC-MS (SIM). Phthalate recovery by this method was 62.5- 140.8%. Detection limits were 0.1-23 ng g^{-1} for each phthalate. In all 63 samples, DEHP was present at the highest level among all phthalates in the range 10-4400 ng g^{-1}.

High concentrations of DEHP (5990 ng g^{-1}) was also found in baby food used in quality assurance work. The source of contamination was the PVC-tube used during production. The analysis of 4-nonylphenols (4-NPs), PCB congeners and phthalates in soils, mesophilic anaerobially digested dewatered (MADD) sewage sludge, and MADD sludge-amended soil were studied by Gibson *et al.* (2005). The samples were soxhlet-extracted. After evaporation, the analytes were separated into two fractions on a cyanopropyl SPE cartridge. This cartridge was chosen because it allowed separation of PCBs from phthalates and NPs, and because better recovery of 4-NPs was possible from this cartridge compared to other similar materials. The NPs/phthalate fraction was analysed by GC-MS directly. The method was successfully validated and then used for routine analysis, where average recoveries of the surrogate standards were 65±140% for phthalate esters.

1.4.6.4 Solid phase microextraction (SPME)

A direct derivative of SPE is solid phase microextraction (SPME), in which a fused-silica micro fibre supports a minute quantity of polymeric extraction phase, where analytes are adsorbed (Buldini *et al.*, 2002). Solid phase microextraction was introduced as a solvent-free sample preparation technique. It is based on SPME is based on the partition of the analyte between the extraction phase and the matrix (Theodoridis *et al.*, 2000). The basic principle of this approach is to use a small amount of the extracting phase, usually less than 1 μL. This technique

combines extraction, concentration and sample introduction in one step. The extracting phase can be either high molecular weight polymer liquid, similar in nature to stationary phase in chromatography, or it can be a solid sorbent, typically of a high porosity to increase the surface area available for adsorption (Pawliszyn, 1999). Although suitable for the analysis of phthalate esters in water (Kelly and Larroque, 1999; Peñalver *et al.*, 2000; Luks-Betlej *et al.*, 2001), sediments and sludge, the direct SPME method cannot be applied to complex matrices such as milk and other dairy products which often contain fat and other biological components (Feng *et al.*, 2005).

SPME has been used as sample preparation in different fields, including environment, food, natural products and pharmaceuticals (Alpendurada, 2000). Until now, sampling by SPME has been used only in some cases for the determination of phthalates (Luks-Betlej *et al.*, 2001). It is usually preferred for the separation of phthalates from water samples followed by GC (Peñalver *et al.*, 2000; Sverdrup *et al.*, 2000 and Peñalver *et al.*, 2001). Different fibers were investigated. A polyacrylate fiber coupled to GC-MS has been used for the pre-concentration of six phthalates and an adipate ester from tap, commercial, industrial and river water samples (Peñalver *et al.*, 2000). It shows linearity was good in the range from 0.05 to 25 mg L^{-1} for most of the analytes with determination coefficients higher than 0.9949 in full scan acquisition mode. The LODs of six phthalate esters were between 0.006 μg L^{-1} and 0.1 μg L^{-1}. The repeatability and the reproducibility of the method, expressed as a percentage of RSD were between 4.2% and 14.2% for repeatability and between 7.8% and 17.4% for reproducibility. However, a comparative study using five fibers for the separation of six phthalates from water showed that a 65-μm polydimethylsiloxane-divinylbenzene (PDMS-DVB) fiber provided the best results, better than other fibers coated with polyacrylate or Carbowax (Peñalver *et al.*, 2001).

Sol-gel method has also been applied to the preparation a polyethylene glycol (PEG) fiber, which has been shown to be useful for the determination of a standard mixture of seven phthalates (Wang *et al.*, 2000). The synthesized 5, 11, 17, 23-tetra-*tert*-butyl-25, 27-diethoxy-26, 28-dihydroxycalix [4] arene (C[4]) blending with hydroxy-terminated silicone oil (OH-TSO) coated SPME fibers (C[4]OH-TSO) showed good selectivity and sensitivity to high boiling point compounds (phthalates), polar (aromatic amines), and nonpolar (benzene derivatives,

polycyclic aromatic hydrocarbons), since the extraction equilibria were reached fast. The detection limits were quite low and the linear ranges were pretty broad for all analytes (Li *et al.*, 2004).

This sol-gel-coated C[4]/OH-TSO fiber was employed in determining the contents of eight phthalate esters plasticizers in blood bags, transfusion tubing, food packaging bag, and mineral water bottle by ultrasonic solvent extraction combined with SPME-GC (Li *et al.*, 2004). The fiber provides good sensitivity and selectivity to the tested compounds. Owing to its high thermal stability (380 °C), the carryover effect that often encountered when using conventional fibers can be reduced by appropriately enhancing the injector temperature. The method showed linear response over two to four orders of magnitude with correlation coefficients (r) greater than 0.996, and limits of detection (LOD) ranged between 0.006 and 0.084 $\mu g\ L^{-1}$. The relative standard deviation values obtained were ≤ 10%. DEHP was the sole analyte detected in these plastics and recoveries were in the ranges 95.5-101.4% in all the samples.

1.4.6.5 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is another technique for solid sample extraction (Gómez-Hens and Aguilar-Caballos, 2003). Supercritical fluid extraction of food has been used for many years on an industrial scale, but it has not been applied to analytical scale sample preparation until recently (Buldini *et al.*, 2002). SFE has also been frequently chosen for the separation of phthalates from different solid samples (Messer and Taylor, 1996; Marín *et al.*, 1996; Albert, 1997 and Bautista *et al.*, 1999). As has been known, recoveries obtained by SFE change dramatically with the pressure and temperature. Thus, a recovery of 100% was obtained for DBP and DIOP from a contaminated soil (Bautista *et al.*, 1999), but the values obtained for DEHP and DBP in the analysis of PVC samples by off-line SFE-GC-FID (Marín *et al.*, 1996) were only 33.4-41.3%, respectively. Supercritical fluids offer considerable advantages as extraction solvents in food analysis. The extracts are cleaner than those obtained with organic solvents and can be obtained minimising thermal degradation. No concentration step is needed prior to chemical analysis. In

spite of the demonstrated advantages and of its considerable industrial application in specific separations, this technique has failed to become a mainstream separation tool because the selection of supercritical fluids and modifiers is largely empirical, due to the existence of very little analyte solubility data, and, in addition, the interactions between supercritical fluid target analytes and sorptive sites on food are still poorly understood (Buldini *et al.*, 2002).

1.4.6.6 Microwave-assisted extraction (MAE)

Microwave-assisted extraction has also been widely used in analytical laboratories for the extraction of organic pollutants including phthalate esters from solid samples (Vandenburg *et al.*, 1997; Bartolome *et al.*, 2005). MAE is a process of using microwave energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent. The ability to rapidly heat the sample solvent mixture is inherent to MAE and the main advantage of this technique. Microwave-assisted extraction has been used for the extraction of phthalate esters in sediment samples by means of a closed microwave system. The filtered extract was further fractionated in two groups using Florisil® cartridges. All the compounds were analysed by gas chromatography mass spectrometry (GC-MS). It found LOD of DEHP ranged from 10-17 mg kg^{-1} (Bartolome *et al.*, 2005). Microwave-assisted solvent elution has also been proposed in SPE for elution of phthalate esters and other pollutants from C_{18} membrane disks during microwave irradiation from a microwave extraction system as an alternative to conventional elution under an applied vacuum, although recoveries are only in the range 70-86% (Chee *et al.*, 1996). The elution of analytes from the solid sorbents normally takes place by organic solvents under an applied vacuum.

The application of microwave radiation to the extraction of phthalate and adipate esters from PVC plastics was described and extracts were measured by gas chromatography with flame ionization detection (GC-FID). The recoveries were found in the range 78-96% (Cortazar *et al.*, 2002). Microwave assisted extraction has been compared to conventional soxhlet and ultrasonic extraction by Chee *et al.* (1996). In this study, a microwave-assisted solvent elution

technique was developed for the elution of polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, polychlorinated Biphenyls, organophosphorus pesticides, fungicides, herbicides and insecticides and phthalarte esters from C_{18} membrane disks during microwave irradiation from a microwave extraction system (MBS). Recoveries 68-90% were obtained for almost all of the organic pollutants under studied. Lower consumption in the use of organic solvent, lesser sample preparation and larger sample throughput are the main advantages over the conventional liquid-liquid extraction.

The advantages of MAE are the reduction of extraction time and solvent usage (Wang and L. Weller, 2006). Samples can be rapidly heated and several samples can be extracted simultaneously. The sample can be contained in a pressure-resistant vessel with safety valves. The solvent can therefore be heated above its normal boiling point. Another advantage compared with soxhlet extraction is that any composition of solvent mixtures can be used. During soxhlet extraction the solvent is the vapor condensate, which will only have the same composition as a mixture of solvents if an azeotropic mixture is used (Vandenburg *et al.*, 1997). MAE allows a larger sample throughput, but like SFE it is expensive and not very widespread. In addition, MAE limits contamination or absorption from the vessel, due to direct heating of the sample. The technique suffers from the disadvantages of using only microwave transparent materials for vessels (Buldini *et al.*, 2002).

From the literature reviews, the analysis of phthalate and adipate esters in packaged food is important for food safety because it has some impacte on human's health. An appropriated sample preparation method for investigating and evaluating these compounds was needed. Therefore, a simple and cost-effective method by ultrasonic extraction followed by the clean up step with SPE using Florisil cartridge prior to analysis by gas chromatography with flame ionization detector were studied. This work focused on the sample preparation for the analysis of phthalate and adipate esters contaminated in packaged food. Both GC-FID and sample preparation conditions were optimized for the best analysis performance. The method were also validated and applied to the quantification of these compounds in real sample.

1.5 Objectives

The objectives of this work are the study of an appropriate sample preparation technique and qualitative and quantitative analysis of trace phthalate and adipate esters contaminated in packaged food using gas chromatography with flame ionization detector (GC-FID).