The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

DETERMINATION OF SIX PHTHALATES IN POLYPROPYLENE CONSUMER PRODUCTS BY SONICATION-ASSISTED EXTRACTION/GC-MS METHODS

(Penentuan Enam Ftalat dalam Produk-Produk Konsumer Polipropilena dengan Kaedah Ekstraksi Bantuan-Sonikasi/GCMS)

Chong Kian Wei*, Loke Chui Fung and Michelle Pang

Chemistry and Biology Division,
School of Arts and Science, Tunku Abdul Rahman College,
Jalan Genting Kelang, Setapak, 53300 Kuala Lumpur, Malaysia

**Corresponding author: chongkw@mail.tarc.edu.my*

Abstract

Studies on determination of six kinds of phthalates, i.e. dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP), in three kinds of plastic containers for food use, including food container, instant noodle cup and snack container, by gas chromatography in combination with mass spectrometry detector (GC-MS) in electronic ionization mode with selected-ion monitoring (SIM) acquisition method (GC-MS(EI-SIM)) have been carried out. Extraction, clean-up and analysis methods have been developed and optimized. Determination of samples were performed after sonication-assisted extraction with 1:9 toluene and dichloromethane, clean-up with Bio-Beads S-X8 gel-permeation column and analyzed by GC-MS methods. The characteristic ions, 163, 194 for DMP; 149, 177, 222 for DEP; 149, 233, 251 for DBP; 91, 149, 206 for BBP; 149, 176, 193 for DEHP; 149, 167, 279 for DNOP were chosen for quantitative studies. These techniques are possible to detect phthalates at the level of 1-70 mg/kg. The overall recoveries were 79.2-91.1% with relative standard deviation (R.S.D.) values at 3.1-11.3%. Only DEHP was detected in the studied samples.

Keywords: Phthalates; Polypropylene Products; Sonication-assisted extraction; GC-MS (EI-SIM); Recoveries

Abstrak

Pengajian penentuan enam jenis ftalat, iaitu dimetil ftalat (DMP), dietil ftalat (DEP), dibutil ftalat (DBP), benzyl butyl ftalat (BBP), di-(2-ethylhexyl) ftalat (DEHP), dan di-n-oktil ftalat (DnOP), dalam tiga jenis bekas plastik untuk kegunaan makanan, termasuk bekas makanan, cawan mee segera dan bekas makanan ringan, dengan menggunakan kromatografi gas bergabung dengan pengesan spektrometer jism (GC-MS) dalam mod pengionan elektronik bersama-sama kaedah pengambilalihan (GC-MS (EI-SIM)) pemantauan ion-selektif (SIM) telah dilakukan. Kaedah ekstraksi, pembersihan dan analisis telah dibangunkan dan dioptimumkan. Penentuan sampel dijalankan setelah ekstraksi bantuan-sonikasi dengan menggunakan pelarut toluena dan diklorometana 1:9, pembersihan dengan turus perembesan-gel S-X8 Bio-Beads dan dianalisis dengan kaedah GC-MS. Ciri ion-ion, 163, 194 untuk DMP, 149, 177, 222 untuk DEP, 149, 233, 251 untuk DBP, 91, 149, 206 untuk BBP, 149, 176, 193 untuk DEHP, 149, 167, 279 untuk DNOP telah dipilih untuk kajian secara kuantitatif. Teknik ini boleh mengesan ftalat pada paras 1-70 mg/kg. Pemulihan keseluruhan adalah 79.2-91.1% dengan nilai sisihan piawai relatif (SPR) sebanyak 3.1-11.3%. Hanya DEHP dikesan pada sampel yang dikaji.

Kata kunci: ftalat; produk polipropilena; exstraksi bantuan-sonikasi; GC-MS(EI-SIM); Pemulihan

Introduction

Nowadays people are easily exposed to phthalates (phthalic acid esters, PAEs) particularly infant. Besides having carcinogenic and estrogenic effects to human, some of the phthalates are toxic and able to induce asthma [1]. The exposure mainly due to the characteristic of phthalates, which is not chemically bound to the polymeric material, therefore; can leach to the environment. Infants have the highest tendency to expose to phthalates because they tend

to put their toys or pacifiers, which may contain phthalates into their mouth. Phthalates in toys which are specifically designed to be chewed by infant may leach into their saliva and then ingested into their stomach. Although the leached phthalates in the saliva are low, the amount is high enough to cause adverse effects to the infant such as liver, reproductive tract and kidney disorders. Cancer is the adverse effect for more serious cases [2]. Due to these effects, many countries in Europe such as Austria, Denmark, Finnish, Greece and Danish had banned the use of phthalates additives in various soft PVC toys and childcare products for children under three years old at the end of the 1990s. Other countries such as Finnish and Danish had stipulated a permissible limit for each relevant phthalates in these products, which should not more than 0.05% [3,4]. Phthalates are widely used as plasticizers in the manufacturing of plastics especially for food packaging. It was suspected to be the endocrine disrupter agents and has estrogen-like structure which is able to displace the estrogen activity [5]. This will further affect the hormone growth and produce breast cancer cells. Human beings have high possibility to be exposed to phthalate esters since these compounds are widely used in the production of agricultural, industrial and household detergents. Dibutyl phthalate (DBP) tends to impair androgen-dependent development for the male reproductive system [6] and inhibits the production of testosterone. This phthalate can be rapidly absorbed into the systemic circulation and spread through the body in a short time. Mono-2-ethylhexyl phthalate, which is one of the urinary metabolites, was detected in the urine content of the workers, who were exposed to high concentration of diethyl phthalate (DEP), dibutylphthalate (DBP), and di(2-ethylhexyl) phthalate (DEHP) mono-2-ethylhexyl phthalate in several PVC production factories. This causes the workers have lower concentration of testosterone compared to the other normal individuals [7]. The US Food and Drug Administration's Center for Devices and Radiological Health and Health Canada have reported the risk assessment of DEHP that migrated from PVC medical devices in hospitalized patients [8].

The analysis of polymer can be considered as two-stage procedure which includes the extraction of the additives from the polymer followed by their identification and quantification. Conventionally, additives in polymer samples are extracted by Soxhlet liquid extraction method. The disadvantages of this method are large volume of solvent and bigger amount of sample are required, long extraction time, contamination and loss of some of the analytes in the preconcentration steps [9]. During the last few years, several methods were proposed for the determination of phthalates by gas chromatography (GC) and high performance liquid chromatography (HPLC) preceded by different preconcentration techniques such as liquid–liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME) [10]. SPME has important advantages over conventional extraction techniques because it is solvent free, fast, portable and easy to be used. However, the fiber used in this method has limited life time due to the fragility and degradation. Therefore, liquid-phase microextraction (LPME) is developed as an alternative extraction technique, which uses only a few microliters of organic solvent. The advantages of using LPME are fast, inexpensive and involving very simple equipments [11]. Supercritical Fluid Extraction (SFE) is a relatively new sample preparation method. An additional advantage of SFE is its selective extraction which can be performed by changing conditions, temperature and density of the fluid [9] with the condition that the liquid solvent must be compatible with the analyte. Gel permeation chromatography (GPC), which is able to separate the macromolecules from the smaller molecules with the use of porous gels or rigid inorganic packing particles is gaining attention recently. According to Kostanski et al. [12], GPC could reproducibly and accurately provide molecular weight distribution and molecular weight averages for linear homo- and uniform composition copolymers under a proper calibration.

In this paper, the studies of gas chromatography in combination with mass spectrometry detector (MSD) for the determination of six kinds of phthalates, i.e. dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP), in plastic products for food use have been reported. A gel permeation chromatography technique was developed, which was able to separate the phthalates from the dissolved polymer. Three kinds of plastic containers for food use, including food container, instant noodle cup and snack container were tested. Approximate two to three characteristic ions of each phthalate were selected for quantitative studies. The method was evaluated by investigating the accuracy and precision of the spiked samples and the developed method was applied for the determination of phthalates in three kinds of real samples.

Experimental

Apparatus and chemicals

A Model U-2000 UV/Vis spectrophotometer (Hitachi, Japan) was used for photometric measurements. A Spectrum 100 fourier transform infrared (FTIR) (Perkin Elmer, USA) coupled with attenuated total Reflectance (ATR) was used to identify the sample material. The concentration of phthalates was measured using a gas chromatograph (Model 7890A, Agilent Technologies. Inc., USA) equipped with mass spectrometer (Model 5975C), automatic liquid sampler (Model 7683B) and Enhanced Chemstation software (version E.02.00.493). An ultrasonic sonicator (JAC Ultrasonic 2010, Kodo-Tech Research Co. Ltd) was used in sample extraction.

DMP, DEP and DBP with purity of 99% were purchased from ACROS organic-New Jersey, USA. DEHP (99%), DnOP (99%), BBP (98%) and amorphous polypropylene were purchased from ALDRICH Chemistry USA. Analytical grade dichloromethane and toluene were purchased from Fisher Chemicals and SYSTERM®ChemAR and used after undergone distillation. Bio-Beads S-X8 (200-400 mesh size) was purchased from Bio-Rad Laboratories Inc., USA. Chromatographic glass column was purchased from PLT Scientific Private Limited, Malaysia.

Standards and spiked samples

Standards were prepared in a mixture of toluene and dichloromethane at the ratio of 1:9. The stock mixture solution of the six phthalates standards at concentration of 6000 mg/L was prepared and stored at 4 ^{o}C. Suitable working solutions with concentration in the range of 5-35 mg/L were prepared from the stock solution to establish the calibration curves. The calibration curves were plotted by peak area versus concentration. Standards with single phthalate at 5 mg/L were prepared for determination of the retention volume of each phthalate. Three batches of 2 g of polypropylene pellets, previously shown to be free from the target compounds, were spiked with 1-3 mg each of the phthalates for recovery testing.

Determination of retention volume

50 g of S-X8 Bio-Beads was soaked in dichloromethane for overnight before packed into a 700 mm x 25 mm i.d. chromatographic glass column as specified in EPA method 3640A [13]. The retention volumes of polypropylene and each of the phthalates were determined by eluting the 100 mL of spiked solution containing 2 g of polypropylene and 0.01 g of phthalate through the chromatographic glass column containing S-X8 Bio-Beads at 3.125 mL/min using 1:9 ratio of toluene and dichloromethane. Every 10 mL of the eluate was collected and the content of the phthalate in each eluate was determined using UV/Vis spectrophotometer at the range of 190-350 nm. Eluted polypropylene was determined by evaporating the excess solvent on the aluminium foil before weighed with balance. Increase weight of the aluminium foil was used as an indication that the polypropylene has been eluted from the column.

Extraction and Clean Up

To avoid phthalates contamination, all glassware used in the study was washed with acetone and rinsed with dichloromethane before dried at 120 ^{o}C for overnight. To ensure the efficiency of extraction of the phthalates, samples were cut with scissors into small pieces. Approximately 2 g of each sample was weighed accurately and transferred to a glass bottle containing 100 mL of 1:9 ratio of toluene and dichloromethane. The extraction was carried out by sonication at 60 ^{o}C until the plastic sample was fully dissolved. The clean-up of the extract was carried out using a chromatographic glass column containing 50 g of Bio-Beads S-X8. The first 100 mL of the eluate was discarded and the following 150 mL was collected for analysis of phthalates using GC-MS. All blanks, spiked samples and real samples were undergone similar extraction and analysis procedures. The recovery test was repeated three times for each spiked concentration. Before extraction was carried out, all real samples were analyzed by FTIR-ATR to determine the sample material.

Data acquisition and analysis conditions

An overview of GC-MS parameters used in the analysis of phthalates in this study was given in Table 1. The phthalates were separated in a 60 m x 0.25 mm, 0.25 µm, DB-5MS, capillary column (Agilent Technologies) with a carrier gas of ultrapure helium at constant flow rate of 1.0 mL/min. The splitless mode was selected for sample injection and the injector temperature was maintained at 300 ^{o}C. The GC oven temperature was programmed with

an initial temperature at 100 ^{o}C for 1 min, then ramped at 20 ^{o}C /min to 300 ^{o}C and then held at 300 ^{o}C for 20 min. The mass spectrometer was operated in electron impact (EI) mode and in the optimum condition based on the automatic tuning result. The transfer line, ion source and quadrupole mass analyser temperatures were maintained at 325, 230, 150 ^{o}C. A solvent delay time of 6.0 min was selected. The phthalates were determined in both full scan (TIC) and selected ion monitoring (SIM) modes. The presence of the phthalates in the samples and standards was confirmed by the mass spectra obtained from the full scan acquisition mode in the range of 50-600 (m/z). SIM mode signal, which was generated by monitoring two to three fragment ions, was used for quantitative determination of the phthalates in the samples and standards. The monitored ions were as follows with the numbers in brackets were used for identification: DMP: 163, (194); DEP: 149, (177), (222); DBP: 149, (233), (251); BBP: (91), (149), 206; DEHP: 149, (176), 193; DnOP: (149), (167), 279. Enhanced Chemstation software was used for control, operation and data acquisition. The method detection limit for each phthalate was calculated from six replicated measurements of a low concentration spiked standard solution according to the Analytical Detection Limit Guidance from Wisconsin Department of Natural Resources [14].

Results and Discussion

Optimization of clean-up procedure

Dissolved polypropylene in the sample needs to be removed before it can be analyzed using GC-MS. This is because polypropylene, which is a macromolecule with high boiling point, tends to deposit in the injector port liner and can cause carry-over contamination to the second analysis. Consequently, sample clean-up is required to remove the dissolved polypropylene from the sample extract. In this study, polypropylene sample containing phthalates were dissolved in 1:9 ratio of toluene and dichloromethane before eluted through a chromatographic glass column containing Bio-Beads SX-8. The collected eluate was injected directly into GC column for further separation and analysis.

Table 1: Gas chromatographic and mass spectrometric parameters used for analysis of phthalates in three kinds of plastic containers for food use

Parameter	
Injector Port	Splitless Mode
Injection Volume	1µL
Injection Port Temperature	300 ^{o}C
Carrier gas–helium flow	1.0 ml min^{-1}
Column (capillary column)	DB-5MS (5% phenyl, 95% dimethylsiloxane)
Column Diameter	60m × 0.25mm × 0.25 µm film thickness
Oven temperature program	100 ^{o}C for 1 min then 20 ^{o}C min^{-1} to 300 ^{o}C for 20 min
Transfer line temperature	325 ^{o}C
Ion Source Temperature	230 ^{o}C
Quadrupole Temperature	150 ^{o}C
Electron energy	69.922 eV
Ionisation current	34.610
Electronic multiplier potential	1270.588 V

Swollen Bio-Beads powder in dichloromethane was packed into the chromatographic column under pressure produced by vacuum pump. Packed column was cleaned with excessive dichloromethane to remove excessive styrene, which was remained in the Bio-Beads powder during the manufacturing. The eluent in the column was replaced to 1:9 ratio toluene and dichloromethane before a spiked phthalate solution was introduced into the

column. The eluate was collected at approximately 3.125 mL/min and the content of phthalate in the eluate was detected using UV/Vis spectrophotometer. The obtained retention volumes were shown in Table 2. As was shown in Table 2, phthalates, which have higher molecular weight, were eluted from the column earlier than the lower molecular weight phthalates. DMP, which has the lowest molecular weight among these phthalates, was collected in 210 to 220 mL fraction, whereas; DEHP and DnOP, which have the highest molecular weight, were collected in 180 to 190 mL fraction. Poplypropylene was collected in 30 to 80 mL fraction. Therefore, based on this result, first 100 mL of eluate eluted from the clean-up column was discarded and the following 150 mL of the eluate was collected for GC-MS analysis.

Table 2: The retention volumes of each phthalate

Sample	Molecular Weight (g/mol)	Retention Volume (ml)
DMP	194.18	210-220
DEP	222.09	190-200
DBP	278.15	190-210
BBP	312.14	180-220
DEHP	390.28	180-190
DnOP	390.28	180-190
Polypropylene	-	30-80

Optimum of data acquisition for GC-MS analysis

Scan mode was used in the GC-MS analysis of the six kinds of phthalates. Retention times and mass spectra of the standards were used to identify the detected phthalates. Figure 1 and 2 are the chromatograms of the full scan mode (TIC) and selected ion mode (SIM) of a mixture of standard of DMP, DEP, DBP, BBP, DEHP and DnOP at 25 mg/L. As shown in these two figures, six kinds of phthalates were well separated by the GC column used in this study. The sensitivities of detecting phthalates were high in SIM mode, which is attributed to the clear baseline obtained in SIM mode chromatogram (Figure 2). The first three phthalates; DMP, DEP and DBP have higher sensitivity than the last three phthalates; BBP, DEHP and DnOP. This could be attributed to some of BBP, DEHP and DnOP, which are less volatile with higher molecular weight compared to the DMP, DEP and DBP, might have deposited somewhere in the GC-MS analysis system or undergone improper fragmentation in the ion source.

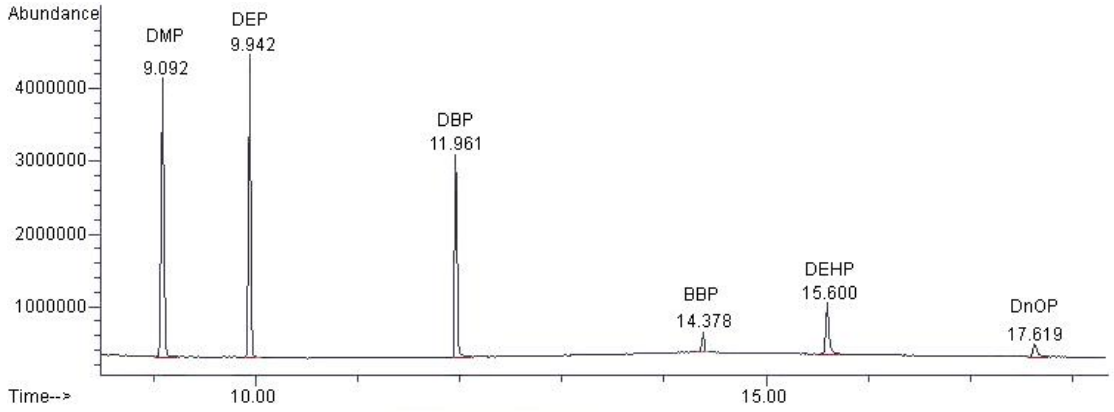

Figure 1: The total ion chromatogram of six phthalates at the concentration of 25 mg/L obtained by full-scan mode (TIC).

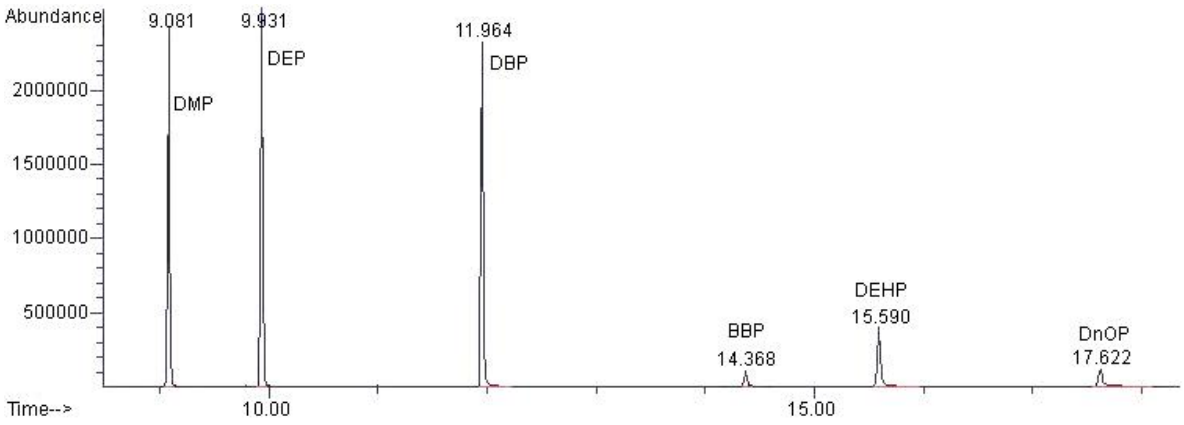

Figure 2: The total ion chromatogram of six phthalates at the concentration of 25 mg/L obtained by selected-ion monitoring (SIM) mode.

Method evaluation

Spiked samples were tested to evaluate the developed method. The developed method was possible to detect phthalates at the level of 1.0-70.0 mg/kg. As shown in Table 3, the recovery data are all within 79.2-91.1% with R.S.D. values at 3.1-11.3%, suggesting that only small quantity of phthalates was lost during the clean-up process.

Real samples

The developed analytical procedure was applied to three real samples of plastic products for food use, i.e. food container, instant noodle cup and snack container, which were bought from the market randomly. All these three samples were scanned with FTIR-ATR to ascertain these samples were made from polypropylene polymer by comparing the IR spectra obtained with the pure polypropylene spectrum. Six kinds of phthalates, i.e. DMP, DEP, DBP, BBP, DEHP and DnOP, were screened and determined by GC-MS-SIM methods. The retention times and mass spectra were used for compound identification, whereas; peak area generated in SIM mode was used for peak quantification using external standard calibration method. The obtained results were listed in Table 4. All of the three examined samples were found to contain DEHP and free from other five phthalates. The detected levels of DEHP in food container and instant noodle cup were complied with the European Community (EC) DEHP maximum permissible level in plastic material, which is not more than 0.1% [15], whereas; DEHP in the snack container is slightly higher than this limit.

Table 3: Average recovery and relative standard deviation for each phthalates

Compound	10 ppm		20ppm		30ppm		Average Recovery	RSDp (%)
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)		
DMP	71.9	6.3	92.9	3.9	72.6	4.3	79.2	5.0
DEP	79.7	2.5	97.1	2.4	96.4	4.3	91.1	3.2
DBP	98.8	2.1	90.7	3.4	74.5	15.0	88.0	8.9
DEHP	91.2	1.3	81.8	12.6	86.4	14.9	86.5	11.3
BBP	78.7	1.5	75.3	11.5	97.6	4.5	83.8	7.2
DnOP	92.9	0.1	97.2	4.5	83.1	2.8	91.1	3.1

Note: RSDp represents Pooled Relative Standard Deviation

Table 4: Determination of phthalates in three kinds of sample by GC–MS–SIM method

Phthalate compound	Detected phthalate level in sample (%)		
	Food Container	Instant Noodle Cup	Snack Container
DMP	ND	ND	ND
DEP	ND	ND	ND
DBP	ND	ND	ND
BBP	ND	ND	ND
DEHP	0.083	0.097	0.127
DnOP	ND	ND	ND

Note: ND represents not detected.

Conclusion

A method for the determination of phthalates in plastic product for food use samples utilizing gel permeation chromatographic clean-up procedure in combination with GC-MS-SIM method was developed. All six phthalates, i.e. DMP, DEP, DBP, BBP, DEHP and DnOP were separated well within 20 min in DB-5MS column without significant interference from the sample matrix. The clean-up with gel permeation chromatography was developed and the retention volumes of each phthalate and polypropylene were determined. First 100 mL eluate, which contained polypropylene, was discarded and the following 150 mL portion was collected for determining of the phthalates content. The developed method was possible to detect phthalates at the level of 1.0-70.0 mg/kg. The overall recoveries are all within 79.2-91.1% with R.S.D. values at 3.1-11.3%. All of the three examined samples were found to contain DEHP but free from other five phthalates.

Acknowledgement

The authors would like to thank Tunku Abdul Rahman College for supporting this research.

References

1. Larsen, S.T., Hansen, J.S., Hansen, E.W., Clausen, P.A., and Nielsen, G.D. (2007). Airway inflammation and adjuvants effect after repeated airborne exposures to di-(2-ethylhexyl) phthalate and ovalbumin in BALB/c mice. *Toxicology*, 235, 119-129.
2. Ema, M. and Miyawaki, E. (2001). Adverse effects on development of the reproductive system in male offspring of rats given monobutyl phthalate, a metabolite of dibutyl phthtlate, during late pregnanacy. *Reproductive Toxicology*, 15, 189-194.
3. Gangi, J.D. (1999). Phthalates in PVC medical products from 12 countries. Greenpeace USA. pp:1-12.
4. Rastogi, S.C., Jensen, G.H., and Worsoe, I.M. (2003). Compliance testing of phthalates in toys. National Environmental Research Institute Ministry of the Environment. pp:11-12.
5. Nakajin, S., Shinoda, S., Ohno, S., Nakazawa, H., and Makino, T. (2001). Effect of phthalate esters and alkylphenols on steroidogenesis in human adrenocorticol H295R cells. *Environmental Toxicology and Pharmacology*. 10, 103-110.
6. Foster, P.M.D., Cattley, R.C., and Mylchreest, E. (2000). Effects of Di-n-butyl Phthalate (DBP) on Male Reproductive Development in the Rat: Implications for Human Risk Assessment. *Food and Chemical Toxicology*. 38, 97-99.
7. Hines, C.J., Hopf, N.B., Deddens, J.A., Silva, M.J., and Calafat, A.M. (2009). Estimated daily intake of phthalates in occupationally exposed groups. *Journal of exposure Science and environmental Epidemiology*, 1-9.

8. Ito, R., Seshimo, F., Miura, N., Kawaguchi, M., Saito, K., and Nakazawa, H. (2005). High-through put determination of mono-and di(2-ethylhexyl) phthalate migration from PVC tubing to drugs using liquid chromatography–tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*. 39, 1036-1041.
9. Marin, M.L., Jimenez, A., Lopez, J., and Vilaplane, J. (1996). Analysis of poly(vinyl chloride) additives by supercritical fluid extraction and gas chromatography. *Journal of Chromatography A*. 750, 183-190.
10. Shen, H.Y. (2005). Simultaneous screening and determination eight phthalates in plastic products for food use by sonication-assisted extraction/GC–MS methods. *Talanta*. 66, 734–739.
11. Farahani, H., Norouzi, P., Dinarvand, R., and Ganjali, M.R. (2007). Development of dispersive liquid–liquid microextraction combined with Gas chromatography–mass spectrometry as a simple, rapid and highly Sensitive method for the determination of phthalate Esters in water samples. *Journal of Chromatography A*. 1172, 105-112.
12. Kostanski, L.K., Keller, D.M., Hamielec, A.E. (2004). Size-exclusion chromatography-a review of calibration methodologies. *Biochemical and Biophysical Methods*. 58, 159-186.
13. EPA method 3640A, Gel-permeation cleanup. 1994, USEPA, Washington DC, pp. l-24.
14. Ripp, J. (1996). Analytical Detection Limit Guidance & Laboratory Guide for Calculating Analytical Detection Limits, Wisconsin Department of Natural Resources, Madison, WI 53707.
15. Official Journal of the European Communities (2002). Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. Official Journal of the European Communities.