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METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF URINARY BISPHENOL A BY GAS CHROMATOGRAPHY - MASS SPECTROMETER (GCMS)

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RINGKASAN : *Kaedah gas chromatograph-mass spectrometric (GCMS) yang sensitif diperbangunkan dan divalidasikan untuk menentukan bisphenol A di dalam sampel air kencing manusia. Bisphenol A diekstrakkan dari air kencing dengan menggunakan kaedah pengekstrakan fasa pepejal (SPE) C18 katrij. Standard surogat yang digunakan ialah bisphenol A-d_{14}. Sebelum pengekstrakan fasa pepejal bisphenol A-d_{14} ditambah dan pengkonjugatan enzim di dalam sampel air kencing dilakukan. Ini adalah untuk menentukan kepekatan keseluruhan bisphenol A (total bisphenol A) yang berasal dari bisphenol A bebas dan bisphenol A-glukuronide. Bisphenol A dianalisa selepas derivatasi menggunakan BSTFA. GCMS kuadrapol detektor di dalam mode SIM digunakan untuk menganalisa bisphenol A. Purata dapatan balik untuk bisphenol A ialah 92.1%, 98.7% dan 94.5% untuk kepekatan 0.1, 0.5, and 0.9 ng/ml masing-masing. Limit kuantitatif untuk bisphenol A di dalam sampel urine adalah 0.05 ng/ml. Bisphenol A keseluruhan (total bisphenol A) di dalam sampel air kencing secara rawak adalah dari <0.05 ng/ml sehingga 5.5 ng/ml (n = 40) dengan purata kepekatan pertengahan ialah 1.3 ng/ml. Bisphenol A bebas hanya dikesan di dalam tujuh sampel air kencing. β-glucuronidase digunakan untuk menentukan kepekatan bisphenol A keseluruhan (bisphenol A total). Sebelum pengekstrakkan, hydrolysis enzim dilakukan untuk membebaskan konjugat. Dua kaedah pengekstrakan yang berbeza dibangunkan untuk menentukan bisphenol A bebas dan bisphenol A keseluruhan.*

ABSTRACT : A sensitive gas chromatograph-mass spectrometric (GCMS) method for the determination of bisphenol A in human urine sample was developed and validated. Bisphenol A was extracted from urine sample by solid phase extraction (SPE) using C18 cartridge. Bisphenol A-d_{14} analogue was used as surrogate standard. Addition of bisphenol A-d_{14} and enzymatic deconjugation of the urine sample was performed prior to solid phase extraction to determine total bisphenol A originating from free bisphenol A and bisphenol A-glucuronide. Bisphenol A was analysed after derivatization with BSTFA using GCMS with quadrapole detector in selected ion monitoring (SIM) mode. Mean recoveries for bisphenol A were 92.1%, 98.7% and 94.5% at 0.1, 0.5, and 0.9 ng/ml concentration, respectively. The quantification limit for bisphenol A in urine was 0.05 ng/ml. Total bisphenol A in urine samples collected randomly from the general public range from <0.05 ng/ml to 5.5 ng/ml (n = 40) with a median concentration of 1.3 ng/ml. Free bisphenol A was detected only in seven urine samples. Determination of total bisphenol A requires the use of β-glucuronidase to enzymatically hydrolyse and release the conjugates before extraction. Two different analyses were conducted to determine free bisphenol and total bisphenol A.

KEYWORDS : Bisphenol A, solid phase extraction (SPE), Gas Chromatograph - Mass Spectrometer (GCMS)

INTRODUCTION

Bisphenol A is an industrial chemical used in the manufacturing of polycarbonate and many types of plastic articles. There is a wide variety of studies available supporting the endocrine activity of bisphenol A. Bisphenol A binds to the estrogen receptor and produces meiotic aneuploidy in the female mouse (Hunt et *al.*, 2003) and increases cell proliferation of the male and female sexual organs (Matthews *et al.,* 2000). Additionally, though still controversial, bisphenol A may induce a decrease of sperm quality in humans. Bisphenol A would not only mimic actions of estrogens, but also as an antagonist of the thyroid hormone action, which might be supported by recent studies where rats exposed to bisphenol A showed an increase in thyroid gland size (Moriyama *et al.,* 2002).

Recent studies have shown that bisphenol A can leach out of certain products, including polycarbonate flasks during autoclaving (Krishnan *et al.,*1993), plastic lining of cans used for food packaging, polycarbonate baby bottles (Tan and Mustafa, 2003b), tableware, white dental fillings and sealants (Olea *et al.,* 1996). Trace levels of bisphenol A have also been found in infant formula powders.

Bisphenol A absorbed by the intestine is glucuronidated in the liver and kidney and excreted as bisphenol A-glucuronide in the urine. Therefore, urinary bisphenol A may be a good marker to estimate exposure to bisphenol A. The conjugation with glucuronide, which is commonly found in rat and human liver microsomes, appears to have a considerable effect on lowering the estrogenic activity of bisphenol A *in vivo* (Elsby *et al.,* 2001). The half life of bisphenol A in blood is short, thus measuring the metabolite of bisphenol A is more desirable for the estimation of bisphenol A exposure in human (Matsumoto *et al.,* 2003). Conjugation with glucuronic acid in the liver is an efficient first-pass-metabolism. Bisphenol A glucuronide formed in the liver is delivered to the blood stream in humans to reach the kidney (Pottenger *et al.,* 2000). When bisphenol A is orally administered to rats, 57-98% is extensively metabolized to glucuronides, 0-4% is sulfate conjugated leaving 1-12% unmetabolized bisphenol A (Knaak *et al.,*1966b).

In recent years, many novel analytical techniques, such as LCMS (Inoue *et al.,* 2000), GCMS (Nakamura *et al.,* 2001) and other spectrometric techniques have been developed for the determination of bisphenol A in water, sediment, tissue and plasma. However, only a few studies have been reported for the quantitative analysis of bisphenol A in urine. Many studies to determine bisphenol A in urine sample were conducted in Japan (Brock *et al.,* 2001). Bisphenol A in urine were determined by negative ion chemical ionization gas chromatography-mass spectrometry (Tsukioka *et al.,* 2003) and by HPLC with multi-channel coulometric electrochemical detector (Ouchi and Watanabe, 2002).

The objective of this study is to improve on the reported GCMS analytical technique for the combined determination of total bisphenol A and free bisphenol A in urine samples. Determination of total bisphenol A requires the use of β-glucuronidase to enzymatically hydrolyse and release the conjugates before extraction. This step is not necessary for the analysis of free bisphenol A. Both determinations were based on Solid Phase Extraction (SPE) using C18 cartridge. SPE was used for sample clean up because it is less tedious, reproducible and only a small amount of solvent is required in the extraction and clean up process. The cleaned samples were derivatised with Bis(trimethylsilyl)tri-fluroacetamide (BSTFA) and the trimethylsilylated form analysed using GCMS.

MATERIALS AND METHODS

Sampling and Storage

Urine samples were randomly collected from 40 healthy volunteers aged 19-45. The volunteers are normal adults who had no known occupational exposure to bisphenol A. All samples were collected in 50 ml beakers that had been baked at 420°C for 3 hours and capped with aluminium sheet. The samples were stored in 30 ml glass bottles with aluminium caps at -20°C until analysis.

Chemicals and Reagents

Bisphenol A and deuterated bisphenol A (bisphenol A-d_{14}) were obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Solvents used for extraction and reconstitution such as acetone, hexane, ethyl acetate, methanol were obtained from Fisher Scientific UK Ltd. and are of HPLC grade. Anhydrous sodium sulfate was also obtained from Fisher Scientific UK Ltd. BSTFA was obtained from Supelco, USA. β-glucuronidase (G7646 Type V11-A) from *E. Coli* was obtained from SIGMA, USA. Phosphate buffer (1 ml, 1 M) was prepared with bisphenol A free water.

Water purified with Milli Q water purification system (Millipore, USA) was filtered through SPE C18 cartridge and the filtrate confirmed bisphenol A free by GCMS. For every batch, a blank bisphenol A free water was analysed to check for possible contamination.

SPE cartridges Chromobond, non polar C18 sorbent (6 ml, 500 mg) were obtained from Supelco, Germany. Glass microfibre filters (GF/A) were obtained from Whatman International Ltd. All glass instruments, except cartridges, were baked at 420°C for 3 hours and covered with aluminium sheets.

Stock standard of bisphenol A and bisphenol A-d_{14} of 100 mg/l in methanol were prepared. The stock solutions were further diluted to 10 mg/l using methanol and stored at 4°C as working standards.

GCMS Analytical Conditions

Quantification of bisphenol A was performed with a Shimadzu QP-5050A gas chromatograph-mass spectrometer with quadrapole detector and analyzed in splitless and selected ion monitoring (SIM) mode. Monitoring ions for derivatised bisphenol A were 357 and 372 while retention time was 9.69 minutes. Monitoring ions for derivatised bisphenol A-d_{14} were 368 and 386 while retention time was 9.51 minutes.

The GC column used was VB-1 (Valcobond, USA) column with a length of 15 m, internal diameter of 0.25 mm and thickness of 0.25 μm. The injection port was set at 310°C while the interface was set at 300°C. GC oven temperature program was as follows: Initial temperature, 120°C, hold for 1 minute; then ramped at 40°C/minute to 184°C, hold for 1 minute; and increased at 1°C/min to 192°C and finally increased at 45°C/min to 280°C. Figure 1 shows the chromatogram of the derivatised bisphenol A-$_{d14}$ and bisphenol A.

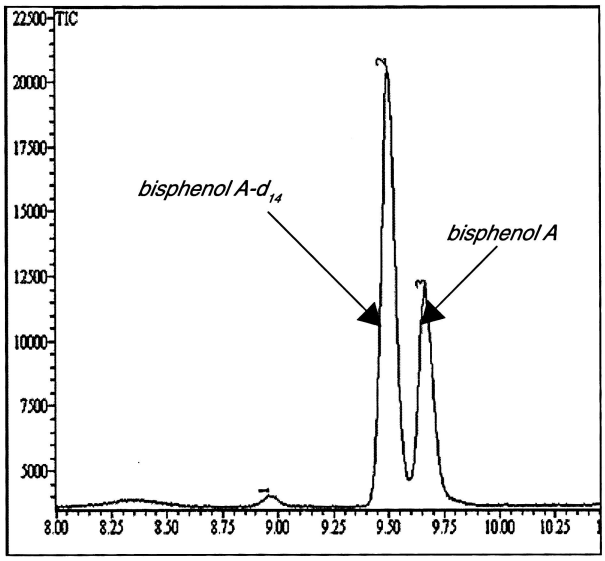

Figure 1. *Chromatogram of BSTFA derivatised bisphenol A-d_{14} and bisphenol A*

Extraction Method for Free Bisphenol A

Prior to the extraction, 10 ml of the urine sample was filtered through glass microfibre filters and spiked with 100 μl of surrogate standard bisphenol A-d_{14} of concentration 100 ng/ml. The amount of urine sample loaded into a C18 cartridge was restricted to maximum of 10 ml to avoid clogging the cartridge.

SPE was carried out according to the following scheme: (a) column conditioning, by sequential addition of 6 ml each of acetone, hexane, ethyl acetate and bisphenol A free water at a flow rate of 1 ml/min; (b) column loading by introducing 10 ml of urine sample to the cartridge at a flow rate of 1 ml/min; (c) washing of the cartridge by rinsing with 12 ml of bisphenol A free water followed by 4 ml of acetone/water (2:3), drying by vacuum pressure for approximately 15 minutes, and final washing with 6 ml of hexane (d) final elution is performed with 6 ml ethyl acetate / hexane (1:4 v/v). The ethyl acetate/hexane eluate was dried with anhydrous sodium sulphate and the solvent fraction completely removed with a stream of nitrogen gas.

Extraction Method for Total Bisphenol A

Bisphenol A-glucuronide in the samples was cleaved enzymatically and both the deconjugated bisphenol A (now as free bisphenol A) and the orginal free bisphenol A are extracted and analysed as the total bisphenol A.

Urine samples (10 ml) were filtered through glass microfibre filters and spiked with surrogate standard bisphenol A-d_{14} (200 μl, 1 ng/ml). The pH of the samples were adjusted to 6.8 by the addition of phosphate buffer (1 ml, 1 M) and incubated with β-glucuronidase (2500 units) overnight at 37°C in a water bath. The procedure for SPE extraction of total bisphenol A was the same as that of free bisphenol A described previously.

GCMS-SIM quantification

BSTFA (100 μl) was added to the dried extract and vortexed for 20 seconds. The mixture was dried in a gentle stream of nitrogen gas and reconstituted with ethyl acetate/hexane (1:4 v/v, 100 μl). 1 μl of the trimethysilylated bisphenol A was analyzed using GCMS-SIM. Quantification of free bisphenol A in the urine sample was based on the area ratio from the calibration curve. The same procedure applies to total bisphenol A.

Bisphenol A Calibration Curve

Standards concentrations of 0.9, 0.8, 0.7, 0.5, 0.2 and 0.1 ng/ml were spiked to six blank urine samples which is free from bisphenol A. Bisphenol A was extracted from the urine sample aliquots according to the procedure and analyzed using GCMS-SIM. The area ratio versus

concentration of extracted bisphenol A was plotted as the calibration curve of free bisphenol A. The curve was linear in the 0-0.9 ng/ml concentration range (r^2>0.993) for free bisphenol A.

The calibration exercise was repeated for higher concentrations of bisphenol A in the range of 0-50 ng/ml and the results were also linear (r^2 > 0.996) for total bisphenol A.

Method validation

The method was validated through a recovery test where standard solutions of bisphenol A were added to three blank urine samples so that they each contained bisphenol A in amounts of 0.1, 0.5 and 0.9 ng/ml respectively. Precision of the method was investigated by analysing the spiked urine samples.

RESULTS AND DISCUSSION

The recovery of bisphenol A was determined by direct comparison of peak areas from spiked versus non spiked samples. The mean recoveries for bisphenol A were 92.1%, 98.7% and 94.5% at the 0.1, 0.5, and 0.9 ng/ml concentrations, respectively. The coefficients of variations (CVs) of bisphenol A recovered for each of the concentrations (n = 5) were below 15%. Intraday and interday extractions showed consistent recoveries with CVs below 10%. Mean intraday accuracies were in the range of 99 - 102% whereas mean interday accuracies were in the range of 94 - 98% (Table 1).

Table 1. Intraday and interday accuracy and precision for bisphenol A in spiked human urine samples

Concentration (ng/ml)	Mean concentration (ng/ml) (n = 5)	Accuracy (%) (n = 5)	Precision (RSD, %)
Intraday			
0.1	0.101	102.4	8.6
0.5	0.497	99.2	5.9
0.9	0.912	101.3	3.3
Interday			
0.1	0.095	95.2	9.9
0.5	0.469	93.8	7.8
0.9	0.884	98.2	6.8

The limit of detection for bisphenol A was 0.01 ng/ml while the limit of quantification was 0.05 ng/ml. This method is sufficiently precise, for the determination of trace amounts of bisphenol A in human urine.

Total bisphenol A in the 40 random urine samples range from non-detected to 5.5 ng/ml with an average value of 1.3 ng/ml. Figure 2 shows concentration of total bisphenol A in 40 random samples from the public. About 82% of the urine samples contained bisphenol A. Approximately 92% of total bisphenol A in the urine samples were below 3 ng/ml. Free bisphenol A was detected only in seven urine samples and the highest was 0.6 ng/ml.

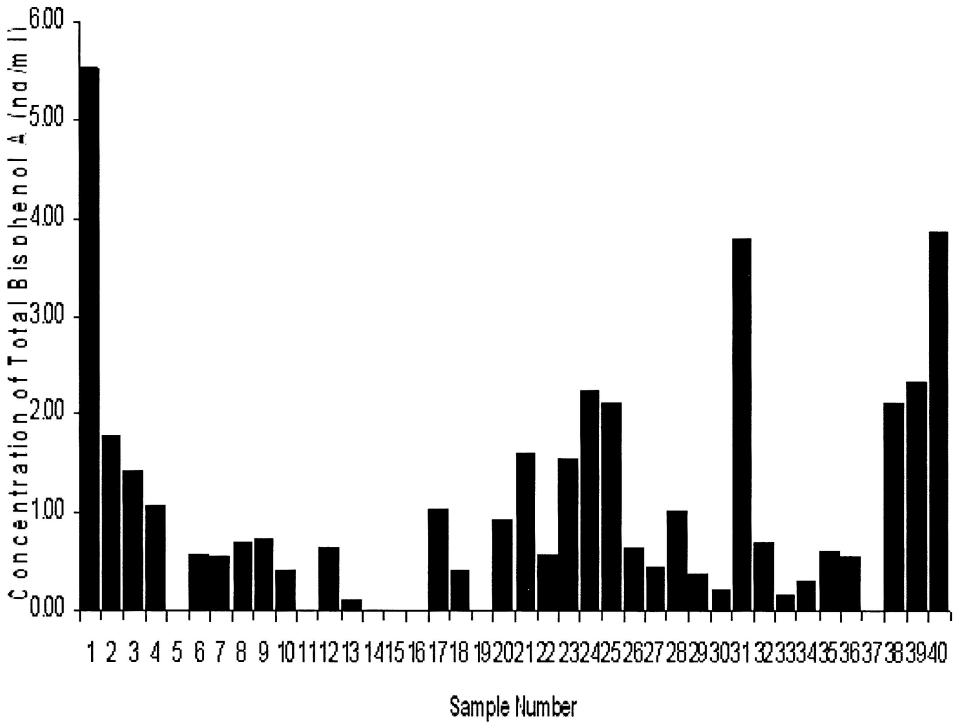

Figure 2. *Concentration of total bisphenol A in 40 random samples from the public*

GCMS-SIM analyses of urine and blood samples for free d_{16} bisphenol A did not give consistent results when identical samples were repeatedly analysed and only small amounts of d_{16} bisphenol A were found in the free form (Volkel *et. al.,* 2002). They also reported that free d_{16} bisphenol A could not be detected after oral administration of d_{16} bisphenol A to humans even at the very low detection limit of 0.2 ng/ml.

The metabolism of bisphenol A has been well characterised in rats with the major metabolite being bisphenol A-glucuronide. After oral administration of ^{14}C bisphenol A (100 mg/kg), most of the label was found in urine and faeces (>91%) with bisphenol A- glucuronide as the major metabolite in urine (>81% of ^{14}C) (Synder *et al.,* 2000). This suggests that total bisphenol A concentration in urine is a more reliable index for biological monitoring of human exposure to bisphenol A.

SPE method was chosen because of fast operation, low consumption of organic solvent, no formation of emulsion and an easily automated procedure compared to liquid-liquid extraction. Milli-Q- purified water, generally considered as contaminant free, was also found to contain trace amounts of bisphenol A of 0.2 ng/ml. By using water free from bisphenol A, a sensitive method can be developed for the determination of trace amounts of bisphenol A in urine.

Water and acetone/water (2:3) mixture were considered as the most satisfactory combination for removing unwanted materials from the SPE column. Acetone/water (2:3) removes impurities, leaving bisphenol A in the column. Proper vacuum and drying were also essential to remove water residues in the SPE column.

BSTFA was chosen as the silylation reagent because of its fast reactivity with bisphenol A, high volatility resulting in less chromatographic interference, low thermal degradation and good solubility in common organic solvents. Bisphenol A was completely and rapidly derivatised when in contact with BSTFA. The derivatised samples presented an improved separation of the analytes during GCMS-SIM analysis, because of their volatility and lower interaction with the stationary phase. The use of the SIM mode during GCMS analysis also contributed to improved selectivity and sensitivity.

Tsukioka and co-workers (2003) detected bisphenol A in all the urine samples collected for their study in Japan in the range of 0.2 to 3.8 ng/ml (n = 6) with the average being 1.6 ng/ml. Ouchi and Watanabe (2002) analysed for levels of free bisphenol A and bisphenol A- glucuronide in urine samples of 48 college-women. Free bisphenol A levels were found to be below the detection limit (0.2 ng/ml) except for one sample at 0.2 ng/ml while bisphenol A -glucuronide was detected in all samples in the range of 0.2-19.1 ng/ml (median 1.2 ng/ml).

There was no gender correlation in the levels of free and total bisphenol A in the urine samples collected from Korean public (Kim *et al.,* 2003). The average bisphenol A in men and women were 2.82 and 2.76 ng/ml, respectively. This is comparable with the findings of this study where about 82% of the 40 urine samples contained bisphenol A. A study on cord blood from Malaysian babies revealed that bisphenol A was detected in more than 80% of these samples (n = 180) and in the range of non detected to 4.0 ng/ml (Tan and Mustafa, 2003a).

It was reported that the urine samples collected in 1992 showed a clear trend between bisphenol A levels with coffee and tea consumption, with the possible implication that the main source of urinary bisphenol A could be from the linings of beverage cans (Matsumoto *et al.,* 1977). Elution of bisphenol A from coatings in cans used for beverages has been confirmed and is estimated at 0-213 ppb with as much as 0-42 μg of bisphenol A eluted from a typical 200 ml can (Kawamura *et al.,* 1999). Can linings could then be one of the main source of bisphenol A contamination to human. Bisphenol A contamination of canned beverages and foods became a serious issue in Japan, and in 1997 most major manufacturing companies in Japan changed the interior can coatings to eliminate or reduce the contamination of bisphenol A. Bisphenol A contamination of human through canned foods has also been identified in relation to canned vegetables, infant formula, and canned fish and meat products (Kawamura *et al.,* 1999).

The levels of bisphenol A in river waters in Japan have been reported as 8 ng/ml. Clark *et al.* (1992) reported that total concentration of alkylphenolic compounds in the drinking water sample was almost 1 μg/l. According to a study done on the Selangor rivers, bisphenol A was detected in the range of 6.33-1588 ng/l (Tan and Mustafa, 2004). This level is high compared to Japan and the U.S.A.

The United States Environmental Protection Agency (EPA) has set a maximum acceptable oral dose for bisphenol A at 0.05 mg/kg body weight/day (EPA 1988). Other regulatory contamination limits for bisphenol A in food are 3 mg/kg in the European Union (EEC 1990) and 2.5 mg/kg in Japan.

Consumption of food contaminated with bisphenol A is a major route of human exposure to the compound. Volkel *et al.* (2002) investigated the metabolism and toxic kinetics of bisphenol A in humans at the low dose exposure. They suggested that glucuronidation of bisphenol A and its rapid excretion is more effective in lowering the estrogenic activity of bisphenol A in humans compared to rats.

CONCLUSION

A specific and sensitive GCMS-SIM method for the determination of bisphenol A was developed and validated, and found suitable for the analysis of a large number of urine samples within a short period of time. The combination of SPE and GCMS-SIM analysis after trimethylsilylation permits the reliable and rapid determination of trace amounts of bisphenol A in human urine samples.

The detection of bisphenol A in more than 80% of public urine samples analysed indicated that the general population (non occupational) are exposed to bisphenol A through various

sources. Total bisphenol A in urine was found to be a better marker to evaluate the exposure of humans to bisphenol A. The use of this method will enable further investigation on the potential correlation between life-style and possibly job function and exposure to bisphenol A.

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REFERENCES

Brock, J.W., Yoshimura, Y., Barr, J.R. (2001). Measurement of bisphenol A levels in human urine. *J. Expo. Anal. Environ. Epidemiol.* **11**: pp 323-328

Clark, L.B., Rosen, R,T., Hartman, T.G. (1992). Determination of alkylphenol ethoxylates and their acetic acid derivatives in drinking water by particle beam liquid chromatography/mass spectrometry. *J. Environ. Anal. Chem.* **147**: pp 167-180

EEC (1990). Commission Directive 90/128/EEC relating to plastics materials and articles intended to come into contact with foodstuffs. pp. 26-46.

Elsby, R., Maggs, J.L., Ashby, J. and Park, B.K. (2001). Comparison of the modulatory effects of human and rat liver microsomal metabolism on the estrogenicity of bisphenol A: implications for extrapolation to humans. *J Pharmacol Exp Ther.* **297 (1)**: pp 103-113

EPA (1988). Bisphenol A reference dose for chronic oral exposure (RfD) in Integrated risk information system (IRIS), a toxicology data file on the National Library of Medicine's (NLM) TOXNET system. On-line http://www.epa.gov/IRIS.

Hunt, P.A., Koehler, K.E. and Susiarjo, M.(2003). Bisphenol A exposure causes meiotic aneuploidy in the female mouse. *Curr. Biol.* **13 (7)**: pp 546-553

Inoue, K., Yoshimura, Y. and Makino,T. (2000). Determination of 4-nonylphenol and 4-octylphenol in human blood samples by high performance liquid chromatography with multi-electrode electrochemical coulometric-array detection. *Analyst.* **125 (11)**: pp 1959-1961

Kawamura,Y., Sano, Y. and Yamada, T. (1999). Migration of bisphenol A from can coating to drinks. *Jounal of Food Hygienic Society of Japan.* **40 (2)**: pp 158-165

Kim, Y.H., Kim, C.S. and Park, S. (2003). Gender differences in the levels of bisphenol A metabolites in urine. *J. Biochem. and Biophys. Res. Commun.* **312**: pp 441-448

Knaak, J.B, Elridge, J.M. and Sullivan, I.J. (1966b). Excretion of certain polyethylene glycol ether adducts of nonylphenol by the rat. *Toxicology and Applied Pharmacology.* **9**: pp 331-340

Krishnan, A.V., Stathis, P. and Permuth, S.F. (1993). Bisphenol A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology.* **132 (6)**: pp 2279-2286

Matsumoto, G., Ishiwatari, R. and Hayna, T. (1977). Gas chromatographic-mass spectrometric identification of phenols and aromatic acids in river waters. *Water Res.* **11**: pp 693-698

Matthews, J.B., Twomey, K. and Zacharewski, T.R. (2000). *In vitro* and *in vivo* interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptor α and β. J. *Chem. Res. Toxicol.* **14 (2)**: pp 149-157

Moriyama, K., Tagami, T. and Akamizu, T., (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J. Clin. Endocrinol. Metab.* ***87 (11)*** : pp 5185- 5190

Nakamura, S., Sian, T. H. and Daishima (2001). Determination of estrogens in river water by gas chromatography-negative-ion chemical-ionization mass spectrometry. *Chromatography A.* **919 (2)**: pp 275-282

Olea, N., Pulgar, R. and Perez P (1996). Estrogenicity of resin-based composites and sealants used in dentistry. *Environ Health Perspect.* **104**: pp 298-305

Ouchi, K. and Watanabe, S. (2002). Measurement of bisphenol A in human urine using liquid chromatography with multi channel coulometric electrochemical detection. *Journal of Chromatography B.* **780**: pp 365-370

Pottenger, L.H., Domoradzki, J.Y. and Markham, D.A., (2000). The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. *Toxicol. Sci.* **54**: pp 3-18

Synder, R.W., Maness S.C. and Gaido K.W. (2000) Metabolism and disposition of bisphenol A in female rats. J. Toxicol. Allpl. Pharmacol. **168**: pp 225-234

Tan, B.L.L and Mustafa, A.M. (2003a). Analysis of selected pesticides and alkylphenols in human cord blood by gas chromatograph- mass spectrometer. *Talanta.* **61** : pp 385-391

Tan, B.L.L and Mustafa, A.M. (2003b). Leaching of bisphenol A from new and old babies bottles, and new babies feeding teats. *Asia Pac. J. of Public Health.* **15 (2)**: pp 118-123

Tan, B.L.L and Mustafa, A.M. (2004). The monitoring of pesticides and alkylphenols in selected rivers in the State of Selangor, Malaysia. *Asia Pac. J. of Public Health.* **16 (1)**: pp 54-63

Tsukioka, T., Brock, J. And Graiser, S. (2003). Determination of Trace Amounts of Bisphenol A in Urine by Negative - Ion Chemical - Ionization-Gas Chromatography/ Mass Spectrometry. *Analytical Sciences* **19** : pp 151-153

Volkel, W., Colnot, T., Csanady, G.A. (2002). Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *J.Chem. Res. Toxicol.* **15**: pp 1281-1287