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ORIGINAL ARTICLE

# Detection of Bisphenol- A in Various Environment Samples Collected from Tamil Nadu, India by Solid-Phase Extraction and GC Analysis

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**ABSTRACT**
*Worldwide, considerable attention has been attracted on wide variety of endocrine compounds, due their efficiency to disrupt the endocrine systems of higher life forms, such as fish, wildlife and humans even at low concentrations. In this study, Bisphenol A (BPA) present in various environment samples of sea water, sediment, river water, soil collected from Tamilnadu, India has been detected by Gas chromatography (GC) analysis using solid –phase extraction method. The results indicated the presence of BPA in all samples that gives the preliminary awareness towards the pollution of BPA in environment. Further studies suggested quantifying the level of BPA exposure in the environment.*
***Keywords:*** *Bisphenol-A, water, soil, sediment, Tamil Nadu*

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## INTRODUCTION

Environment draws great attention worldwide due to the release of various harmful organic pollutants, because these pollutants can eventually become a risk or threat to both animals and humans [1]. Endocrine disrupting chemicals (EDCs) are environment contaminants; interfere with the normal hormone functions in wild life and humans [2]. The wide range of chemicals reported as EDCs includes some phenolic compounds [3]. Among these Bisphenol A (BPA) has been given more attention due to their usage. BPA is mainly used in the production of polycarbonate plastic, epoxy resins and non-polymer additives [4, 5, 6] BPA is also used by manufacturers as an intermediate in the production of adhesives, protective coatings, paints, automotive lenses, protective windows glazing, building materials, compact disks, optical lenses, thermal paper, paper coatings, as a developer in dyes, for encapsulation of electrical and electronic components, as dental sealant and composites [7, 8, 9]. Due to its wide applications and growing demand, it has become one of the highest yielding chemicals in the world [10].

Sewage containing industrial waste is the major source of environmental pollution, including BPA deposition [11]. In India, most of the cities/towns are struggling to treat the sewage as per standard guidelines before disposing in to the environment. Nearly 75% of the sewage wastes are still discharged in to coastal regions and local water bodies without prior treatment [12]. Due to wide range of negative effects created by BPA, it is necessary to detect their presence in environment samples. BPA has been mainly detected in sewage sludge [13, 14], biological matrices [15, 16], air [17], water [18] and soils [19].BPA is also detected in landfill leachates [20], ground waters and agricultural wastes [21]. Although studies reported the presence of BPA in various environments, there is no or little attention so far received in India. Further, published data about on the occurrence of BPA in Indian environment, especially in Tamil Nadu is hardly available.

With this environmental aspect in focus, this study, attempted to detect the occurrence of BPA in various environmental samples in Tamil Nadu, India, using a solid phase extraction method and GC analysis. To our knowledge this is the first report for the simultaneous detection of BPA in various environmental samples collected from Tamilnadu state, India.

## MATERIALS AND METHODS

### Chemicals

BPA (GC grade >99%) was purchased from Sigma-Aldrich (India). Dichloromethane for GC analysis at extra pure grade was purchased from Merck, India. Strata C18- SPE Column obtained from Phenomenex, India. Silica (60-120 mesh) for cleaning of samples was purchased from Sigma. Anhydrous sodium sulphate for drying solvent extract prior to GC analysis was of AR grade, purchased from Rankem, India.

### Sample collection

Seawater was collected from coastal region of Chennai, Tuticorin and Kanyakumari. River water was collected from Amaravathi river, Bhavani river and Shanmuga river. Soil sample was collected form Coimbatore and sediment sample was collected costal region of Chennai. The samples were collected after the northeast monsoon during January and February 2012 in sterile glass containers previously washed with ethanol.

### Solid phase extraction of BPA from environment samples

C18-SPE column was conditioned with 2ml dichloromethane and 2ml sterile distilled water. Sea and river water samples were passed through the column manually and allowed to dry at $40^0$C for 4 hrs. Then column was eluted with 2x 2ml dichloromethane. Eluted dichloromethane was evaporated to 1 ml and subjected to GC analysis. 100g of soil and sediment sample were taken in 500ml Erlenmeyer flask, mixed with 100ml of dichloromethane and kept in a rotary shaker at 150rpm for 24hrs. Sample extracts were passed through 30cm x 20mm chromatographic glass column filled with silica and anhydrous sodium sulphate. Just prior to use, the column containing absorbent was washed with dichloromethane, then sample extract was eluted with additional 20ml of dichloromethane.

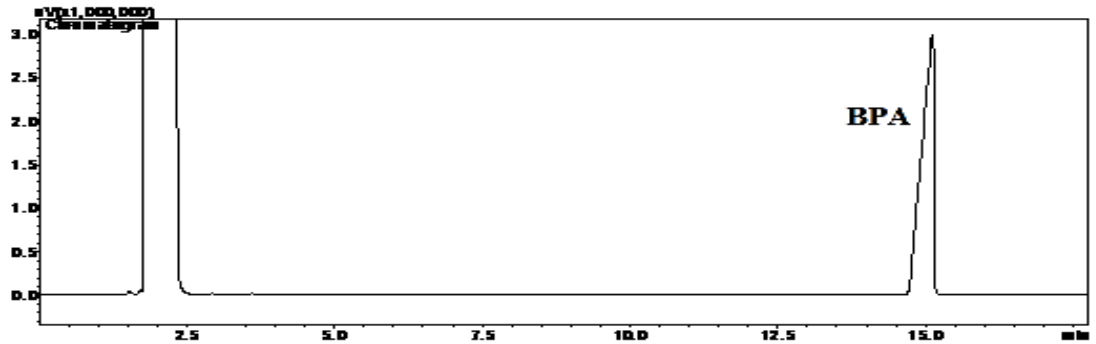
### Gas Chromatography analysis

The eluted sample was concentrated as 1 ml and subjected to GC analysis. Shimadzu-2014AF model with flame ionization detector was used and operating parameters were as follows: Carrier Gas- Ultra Pure Nitrogen, Flow rate of gas- Nitrogen -40ml /min, Flame Source -Hydrogen and Zero air (60ml/min). Injection temperature -275°C Column temperature – 240°C, Detector temperature – 310°C,Sample injection volume- 1µl.

## RESULTS AND DISCUSSION

Analytical methods for the determination of EDCs in various liquid and solid matrices have been developed due to the presence of these substances in environment from various input activities [22]. Based on chemical properties, EDCs tend to bind tightly to sediments and bioaccumulate in aquatic organisms, which contribute to the persistence of these compounds in the environment and requires their determination from several matrices [23, 24]. Because of solubility in water and relatively high vapor pressure of some of EDCs, their degradation products can be transferred to the environment at relatively high concentration through aquatic or atmospheric systems [25]. Sediments or solids are good adsorbents of phenolic contaminants due to their high surface area and surface activity. Because of their importance in monitoring the levels in the environment, the analysis of phenolic compounds in sediment and solid samples has been extensively studied [3]. In this work, different environment samples like seawater, river water, coastal region sediment and soil were collected from different regions of Tamil Nadu, India and used to test the occurrence of BPA.

**Fig. 1: GC chromatogram of BPA standard solution**



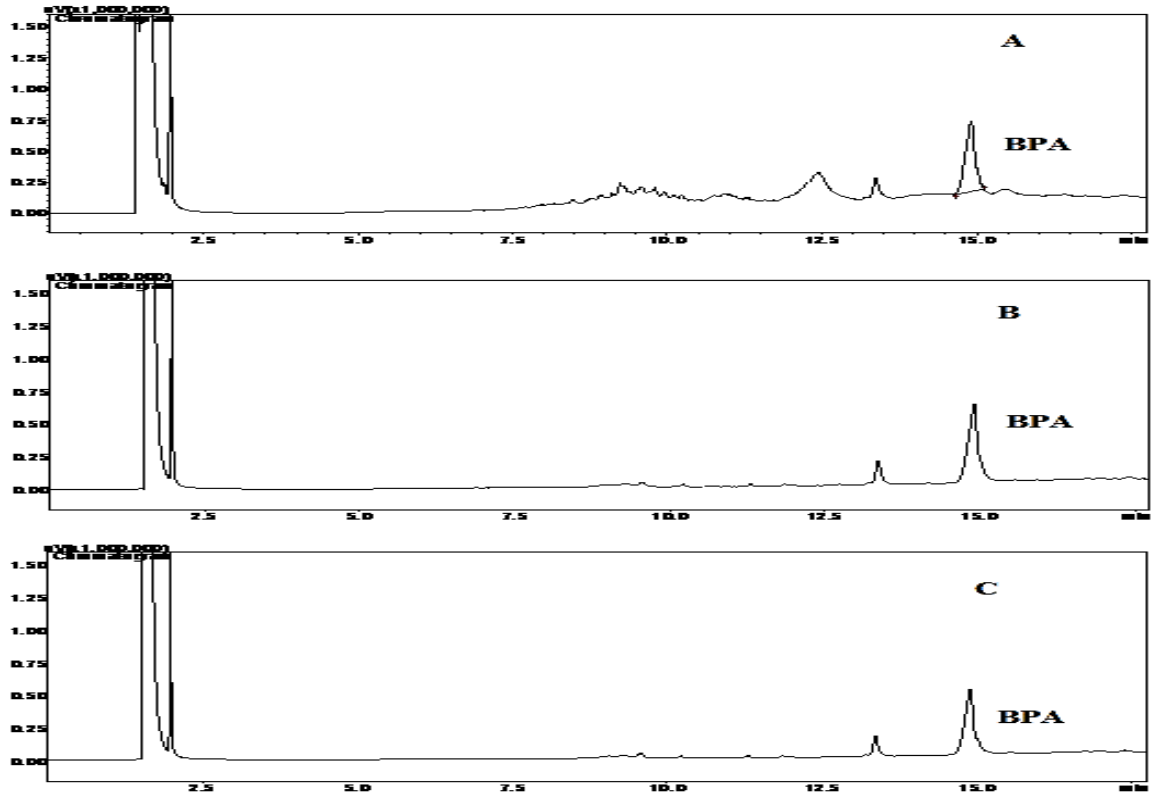



**Fig 2: GC chromatogram of river water samples from Amaravathi (A), Bhavani (B) and Shanmuga (C) river**

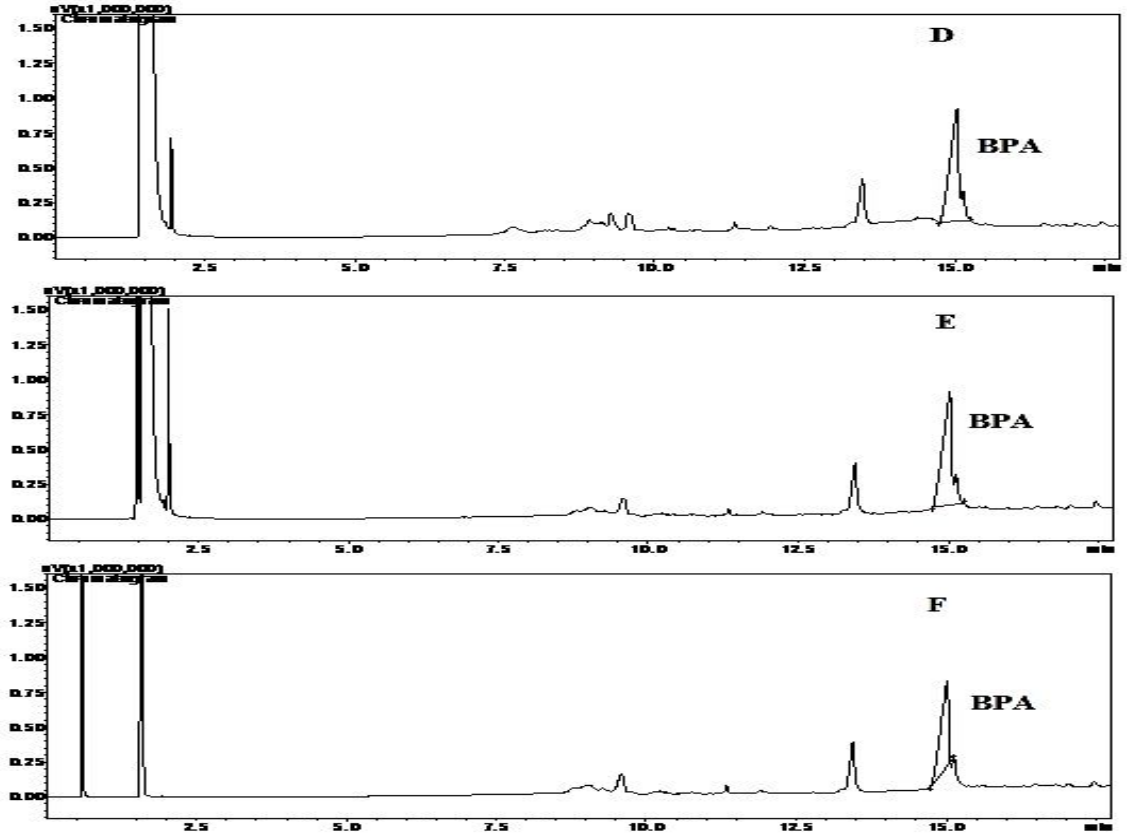



**Fig. 3: GC chromatogram of seawater samples from Chennai (D), Tuticorin (E) and Kanyakuamri (F) coastal region**

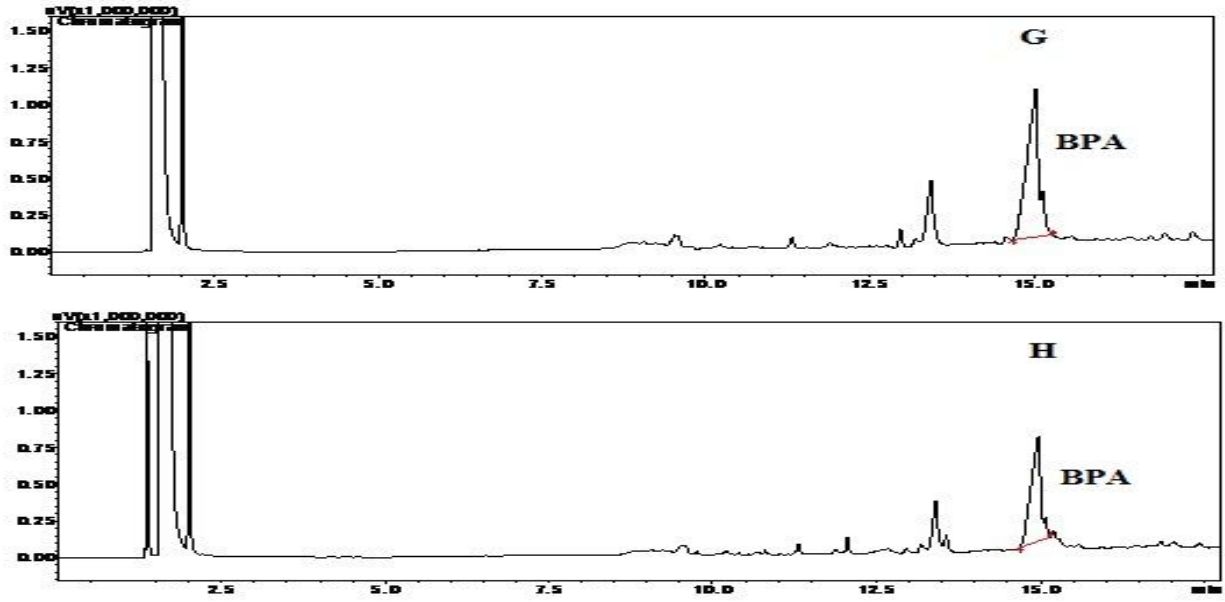



**Fig. 4: GC chromatogram of sediment (G) and soil (H) sample**

Gas chromatography spectrum of BPA standard solution showed peak at the retention time of 15.22min (Fig 1). The peaks observed in the samples at the same retention period may due to the presence of BPA. Chromatogram corresponding to BPA was found in all analyzed samples of sea waters, river waters, sediments and soil and intensity of chromatogram corresponded to the concentration of BPA. Chromatogram represented the ionization of BPA found in Amaravathi, Bhavani and Shanmuga river waters (Fig 2) and measured as 6620921µV, 6684307 µV and 5168157 µV respectively. Ionization of BPA was 6111524µV, 6942463 µV and 5740705 µV in Chennai, Tuticorin and Kanyakumari coastal regions seawaters respectively (Fig 3). Chromatogram of BPA ionization was found as 7572517 µV, 122250103 µV in soil and sediment respectively (Fig 4). The peak area suggested that among other samples used in this study, highest BPA occurrence was found to be in Chennai coastal region sediment and lowest found to be in Shanmuga river water. Similar ionization values were observed between seawaters and river water and it denotes that the occurrence of BPA was more or less similar in all the samples.

Among various sample pretreatment techniques, solid phase extraction (SPE) is the most common technique for environmental water sample pretreatment, because it has many obvious advantages over traditional liquid-liquid extraction (LLE), such as high recovery, high pre-concentration factor, low consumption of organic solvents, simplicity, easy automation and operation and so on [26,27]. With regard to the extraction method and based on the results obtained from previous work, the present study was based on Solid-phase extraction. Various solvents have been used for BPA extraction. Several of these extraction solvents and dichloromethane was chosen as the most adequate solvent relative to other solvents.

Optimal sample cleanup was noted as being dependent on the C-18 SPE cartridge step. However, in our experiments, C-18 SPE cartridge cleanup was beneficial with all environmental samples. C-18 SPE cleanup may be more suitable for sediment samples where the organic compound contamination is low. When target compounds are pre-concentrated, any potentially interfering contaminants will also be concentrated, which are often present in far greater quantities than the target compounds in the sample [28].In our studies, along with peak observed for BPA, additional peaks were also observed due to the high concentration of other chemicals.

Based on different principles many methods have been proposed for the determination of BPA Such as TLC [29], GC [30] or HPLC with conventional ultraviolet detection [31] fluorescence [32] or electrochemical detection [33]. For the determination of mg/l and ng/l concentrations of BPA in environmental samples the most frequently used methods are gas chromatography–mass spectrometry (GC–MS) [33] or high-performance liquid chromatography with ultraviolet (UV), mass spectrometric or electrochemical detection [34]. In the present study GC analysis is used for the detection of BPA in

various environment samples. The volatility and thermal stability of BPA make it suitable for detection by Gas Chromatography [35].

## CONCLUSION

BPA occurrence has been tested in eight environment samples collected from different regions of Tamil Nadu. Solid –Phase extraction method was used for BPA extraction in both liquid and solid samples and GC analysis for detection of the BPA extraction. In all results, the occurrence of BPA was found by the presence of BPA corresponding peak in Gas Chromatography analysis. This preliminary study of BPA analysis in environment samples and results suggested that all places where the samples used in this study were collected were significantly polluted with BPA. The concentration of BPA varied in all the samples due to their geographical location, time zone, geological foundation and possible routes of pollution. This study would help to create and develop awareness among the people on BPA pollution. Since this study was performed on field, it has the potential to initiate other researchers to carry further detailed analysis of BPA in Indian environment.

## REFERENCES


1. Zhao, R. S., Wang, X., Yuan, J. P. & Zhang, L. L. (2009). Solid-phase extraction of bisphenol A, nonylphenol and 4-octylphenol from environmental water samples using microporous bamboo charcoal, and their determination by HPLC. Microchim. Acta., 165 (3-4): 443–447.
2. Nie, Y., Qiang, Z., Zhang, H. & Adams, C. (2009). Determination of endocrine-disrupting chemicals in the liquid and solid phases of activated sludge by solid phase extraction and gas chromatography–mass spectrometry. J. Chromatogr. A., 1216 (42): 7071–7080.
3. Porcel, J. L. P., Parreno, M. M., Soriano, E. M. & Valor, I. (2009). Analysis of chlorophenols, bisphenol-A, 4-tert-octylphenol and 4-nonylphenols in soil by means of ultrasonic solvent extraction and stir bar sorptive extraction with in situ derivatisation. J.Chromatogr A., 1216 (32): 5955–5961.
4. Staples, C. A., Dorn, P. B., Klecka, G. M., Block, S. T. & Harris, L. R. (1998). A review of the environmental fate, effects, and exposures of bisphenol A. Chemosp., 36 (10): 2820–2914.
5. Kuruto-Niwa, R., Tateoka, Y., Usuki, Y. & Nozawa, R. (2007). Measurement of Bisphenol A concentrations in human colostrums. Chemosp., 66 (6): 1160–1164.
6. Grumetto, L., Montesano, D., Seccia, S., Albrizio, S. & Barbato, F. (2008). Determination of bisphenol A and bisphenol B residues in canned peeled tomatoes by reversed-phase liquid chromatography. J. Agric. Food Chem., 56 (22): 10633–10637.
7. Zafraa, A., del Olmoa, M., Suareza, B., Hontoriab, E., Navalona, A. & Vılcheza, J. S. (2003). Gas chromatographic–mass spectrometric method for the determination of bisphenol A and its chlorinated derivatives in urban wastewater. Water Res., 37 (4): 735–742.
8. Furhacker, M., Scharf, S. & Weber, H. (2000). Bisphenol A: emissions from point sources. Chemosp., 41 (5): 751–756.
9. Pulgar, R., Olea-Serrano, M. F., Novillo-Fertrell, A., Rivas, A., Pazos, P., Pedraza, V., Navajas, J. M. & Olea, N. (2000). Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. Environ Health Perspect., 108 (1): 195–202.
10. Gomez, A. B., Rubio, S. & Bendito, D. P. (2009). Analytical methods for the determination of bisphenol A in food. J. Chromatogr. A., 1216 (3): 449–469.
11. Rubin, B. S. & Soto, A. M. (2009). Bisphenol A: perinatal exposure and body weight. Mol. Cell. Endocrinol., 304 (1-2): 55–62.
12. Babu Rajendran, R., Govindaraj, S., Geetha, V., Bhuvaneshwari, R. & Joakim Larsson, D. G. (2011). GC–MS analysis and ecotoxicological risk assessment of triclosan, carbamazepine and parabens in Indian rivers. J. Hazard. Mater., 186 (2-3): 1586–1593.
13. Ying, G., Kookana, R. S. & Ru, Y. (2002). Occurrence and fate of hormone steroids in the environment. Environ. Int., 28 (6): 545-551.
14. Auriol, M., Meknassi, Y. F., Tyagi, R. D., Adams, C. D. & Surampalli, R. Y. (2006). Endocrine disrupting compounds removal from wastewater, a new challenge. Process Biochem., 41 (3): 525-539.
15. Bogers, R., Vries-Buitenweg, S. D., Geuijen, I., Waart, B. V. D., Kuiper, R., Linden, S. V. D., Puijker, L., Murk, A., Burg, B. V. D. & Legler, J. (2007). An *in vitro*/*in vivo* screening assay as a sensitive tool to assess endocrine disruptive activity in surface water. Environ. Int., 33 (3): 292-301.
16. Nelson, J., Bishay, F., Roodselaar, A. V., Ikonomou, M. & Law F. C. P. (2007). The use of in vitro bioassays to quantify endocrine disrupting chemicals in municipal wastewater treatment plant effluents. Sci. Total Environ., 374 (1): 80-90.
17. Schultis, T. & Metzger, J. W. (2004). Determination of estrogenic activity by LYES-assay (yeast estrogen screen-assay assisted by enzymatic digestion with lyticase). Chemosp., 57 (11): 1649-1655.
18. Hernando, M. D., Mezcua, M., Gomez, M. J., Malato, O., Aguera, A. & Fernandez- Alba, A. R. (2004). Comparative study of analytical methods involving gas chromatography–mass spectrometry after derivatization and gas chromatography–tandem mass spectrometry for the determination of selected endocrine disrupting compounds in wastewaters. J. Chromatogr. A., 1047 (1): 129-135.

19. Lagana, A., Bacaloni, A., Leva, I. D., Faberi, A., Fago, G. & Marino, A. (2004). Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. Anal. Chim. Acta., 501 (1): 79-88.
20. Wintgens, T., Gallenkemper, M. & Melin, T. (2003). Occurrence and removal of endocrine disrupters in landfill leachate treatment plants. Water Sci. Technol., 48 (3): 127–134
21. Latorre, A., Lacorte, S. & Barcelo, D. (2002). Presence of nonylphenol, octylphenol and bisphenol a in two aquifers close to agricultural, industrial and urban areas. Chromatogr., 57 (1-2): 111–116.
22. Reemtsma, T. (2003). Liquid chromatogrphy- mass spectroscopy and strategies for trace analysis of polar organic pollutants. J. Chromatogr. A., 1000 (1-2): 477-501.
23. Kang, J. H. & Kondo F. (2005). Bisphenol A degradation in seawater is different from that in river water. Chemosp., 60 (9): 1288–1292.
24. Vandenberg, L. N., Hauser, R., Marcus, M., Olea, N. & Welshons, W. V. (2007). Human exposure to bisphenol A (BPA). Reprod. Toxicol., 24 (2): 139–177.
25. Dachs, J., Van Ry, D. A. & Eisenreich, S .J. (1999). Occurrence of estrogenic nonylphenols in the urban and coastal atmosphere of the lower Hudson River estuary. Environ. Sci. Technol., 33 (15): 2676-2679.
26. Zhou, Q. X., Ding, Y. J. & Xiao, J. P. (2006b). Sensitive determination of thiamethoxam, imidacloprid and acetamiprid in environmental water samples with solid-phase extraction packed with multiwalled carbon nanotubes prior to high-performance liquid chromatography. Anal. Bioanal. Chem., 385 (8): 1520-1525.
27. Zhou, Q. X., Xiao, J. P. & Wang, W. D. (2006a). Using multi-walled carbon nanotubes as solid phase extraction adsorbents to determine dichlorodiphenyltrichloroethane and its metabolites at trace level in water samples by high performance liquid chromatography with UV detection. J. Chromatogr. A., 1125 (2): 152-158.
28. Chu, S., Haffner, G. D. & Letcher, R. J. (2005). Simultaneous determination of tetrabromobisphenol A, tetrachlorobisphenol A, bisphenol A and other halogenated analogues in sediment and sludge by high performance liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A., 1097 (1-2): 25-32.
29. Challa, G. & Hermans, P. H. (1960). Paper Chromatography of Bisphenol A. Anal. Chem., 32 (7): 778-779
30. Brydia, L. E. (1968). Determination of bisphenol A and impurities by gas chromatography of their trimethylsilyl ether derivatives. Anal. Chem., 40 (14): 2212-2215
31. Eckerman, M. L. H. & Laijoki, T. (1988). Determination of oligomer 340 and polyamines in cured epoxy resins by extraction and high-performance liquid chromatography. Analyst, 113 (2): 239-242.
32. Gandara, J. S., Abuin, S. P., Paseiro, P. & Lozano, J. S. (1993). Determination of Bisphenols A and F in Noncured Epoxy Resins by RP-HPLC—Fluorescence Techniques. J. Chromatogr., 31 (11): 450-454
33. Peltonen, K. & Pukkila, J. (1988). Determination of bisphenol A in air by high-performance liquid chromatography with electrochemical detection. J. Chromatogr. A., 439 (2): 375-380.
34. Sajiki, J., Takahashi, K. & Yonekubo, J. (1999). Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. J. Chromatogr. B., 736 (1-2): 255-261.
35. del Olmo, M., Casado, A. G., Navas, N. A. & Vilchez, J. L. (1997). Determination of bisphenol A (BPA) in water by gas chromatography-mass spectrometry. Anal. Chim. Acta., 346 (1): 87-92.