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기체크로마토그래피/질량분석법을 이용한 캔 물질에서의 bisphenol-A 분석

김기철[1], 김양희[1], 최옥경[1], 고환욱[1], 임준래[1],
조규홍[1], 조현우[2], 김혜영[3], 김명수[3], 명승운[3]★
[1]경기도 보건환경연구원, [2]경기대학교 화학과, [3]한국과학기술연구원 도핑콘트롤센터
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Determination of bisphenol-A using GC/MS in Can Materials

Ki-Cheol Kim[1], Yang-Hee Kim[1], Ok-Kyung Choi[1], Hoan-Uck Ko[1], Jun-Rae Yim[1], Kyu-Hong Choi[1], Hyun-Woo Cho[2], Hye-Young Kim[3], Myungsoo Kim[3], Seung-Woon Myung[3]★
[1]Kyonggi-do Institute of Health & Environment Research, 324-1 Pajang-dong, Jangan-ku Suwon, Kyonggi-do 440-290, Korea
[2]Department of Chemistry, KyongGi University, Suwon, Kyonggi-do 442-760, Korea
[3]Doping Control Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 136-791, Korea
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요 약 : 비스페놀-A는 epoxy resin, polycarbonate, polyester-styrene resin 등의 플라스틱 제조에 사용되는 물질로써, 기체 크로마토그래피를 이용하여 간단하며 빠르고 감도 좋은 분석방법을 연구하였다. 비스페놀-A를 acylating 시약을 사용하여 크로마토그래피의 성질에 적합하도록 유도체화 한 후 SIM(selected ion monitoring) mode를 이용하여 GC/MS(gas chromatography/mass spectrometry)로 분석하였으며, 본 연구 결과 캔 물질에서 비스페놀 A는 0.11~11.40 ㎍/can 이 검출되었다.

Abstract : A new simple, rapid and sensitive gas chromatographic technique for the determination of bisphenol-A in can materials, which is the major material of epoxy resin and polycarbonate polymer, is proposed. This method is characterised by derivatization of the bisphenol-A with a acylating reagent forming the acetate derivative to optimize the chromatographic property. The detection of bisphenol-A is performed based on GC/MS (gas chromatography/mass spectrometry).

Several beverages were analyzed by the proposed method for the determination of bisphenol-A. Bisphenol-A was assayed the range of 0.11~11.40 ㎍/can from the can materials.

Key words : endocrine disruptors, bisphenol-A, GC/MS, can materials

1. Introduction

Bisphenol-A is a common industrial chemical used in various food packaging[1]. Contact between the food and inner coated can lead to the migration of bisphenol-A residues in the product[2,3].

Recently, the preliminary data reported in the literature[4-7] have provided suggestive evidence that bisphenol-A may have weak estrogenic activity, although

★ Corresponding author
Phone : +82-(0)2-958-5104, Fax : +82-(0)2-958-5059
E-mail : swmyung@kist.re.kr

their effects in the animal and human are far from clear. The effect of bisphenol-A on health has become a controversial issue. These are continuing worldwide concern about pesticides and other chemicals that disrupt the endocrine system as reproductive abnormalities in wildlife, increasing the breast cancer in women and decreasing sperm counts in men.

So far, analysis of bisphenol-A has mainly been accomplished by different chromatographic methods, such as TLC[8], GC[9], HPLC with conventional UV detection[10] or fluorescence[11] and GC/MS[12].

In this study, rapid, sensitive and reliable methods by gas chromatography/mass spectrometry are developed for the determination of bisphenol-A. The samples are extracted with dichloromethane, and the extract was esterificated with acetic anhydride and finally determined by GC/MS. In several cases, GC/MS has used to assay some bisphenol-A from the various food and food packaging.

2. Experimental

2.1. Chemicals

All organic solvents (dichloromethane, acetone, ethylacetate) used were of analytical reagent grade and obtained from J.T.Baker (U.S.A.). Bisphenol-A (purity > 99.0%) was purchased from Wako Chemical Co. (Japan). Deuterated bisphenol-A (bisphenol-A-d_{16}) was used as internal standard, which was purchased from Aldrich (WI, USA). Acetic anhydride (Sigma, U.S.A.) was used as a derivatizing reagent of bisphenol-A.

2.2. Extraction of bisphenol-A in epoxy can coating materials

After samples were filled with 50 mL of dichloromethane, the samples were sonicated in an ultrasonic water bath for 20 min. The extraction procedure was repeated twice with the same volume of fresh dichloromethane. After a series of filtrations and washings, the combined extracts of dichloromethane were concentrated under nitrogen gas stream. The extract residues were dissolved with 1 mL of aceton for the esterfication, and 0.2 ㎍ of the deuterated bisphenol-A (internal standard) was then added, .

2.3. Derivatization of bisphenol-A

Into 1 mL of acetone containing the extracts, 500 uL of saturated sodium hydroxide solution and 500 uL of acetic anhydride were added. The samples were heated at 80℃ for 10 min and cooled at room temperature for the GC/MS analysis.

For optimization of the derivatization conditions, various reactions were attempted. Six different amount of derivatizing reagents were evaluated in this study. Derivatization was also performed at various temperatures and reaction times. The optimum derivatizing condition was obtained from the constant sensitivity for the GC/MS.

2.4. Calibration curves

Calibration curve was obtained from spiked sample in the range of 0.5-16 ng and added 0.3 ug of the deuterated bisphenol-A. The spiked samples were treated according to the described method above. The number of each spiked samples were three. The residue was dissolved in 1 mL of ethylacetate and the equation for the quantification was generated by the least-square linear regression analysis.

2.5. Instrumentation and chromatographic conditions

For GC/MS analysis, a HP6890 gas chromatograph/ HP5973 mass selective detector with an electron impact(EI) of 70 eV as ionization source and quadrupole mass filter is used. The column was an HP-5 MS column (30 m×0.25 mm i.d., 0.25 ㎛ film thickness) with helium carrier gas and the flow rate was set 1.0 $mLmin^{-1}$ and split ratio was 1:5. The oven temperature was as follows: 150℃ for 1 min; increased to 270℃ at a rate of $10℃min^{-1}$; final holding time 5 min. Quantitative analyses were based on the integrated SIM (Selected Ion Monitoring) mode. The ions monitored were m/z 213 for bisphenol-A, and m/z 224 for the internal standard.

3. Results and discussion

The bisphenol-A in the GC analysis has shown the

asymmetric peaks (tailing) due to its polarity, and unpredictable reproducibility of repeat analyses[12]. It was found that the presence of co-extracted materials, which are injected into the hot injection port, caused "ghosting" which led to the unpredictable data[12]. The acetyl derivative of bisphenol-A produced the good chromatographic properties without ghost peaks and peak tailing. In addition, the reproducibility and repeatability were further.

To obtain an optimum condition for the analysis of bisphenol-A, the effects of several parameters were investigated for the concentration of 10 μg/mL. For the amount of the acetic anhydride, the derivatizing efficiency was increased with the added amount of acetic anhydride, retaining the constant peak area at the amount more than 500 uL, when the reaction temperature is 80℃ and the derivatizing time is 10 min.

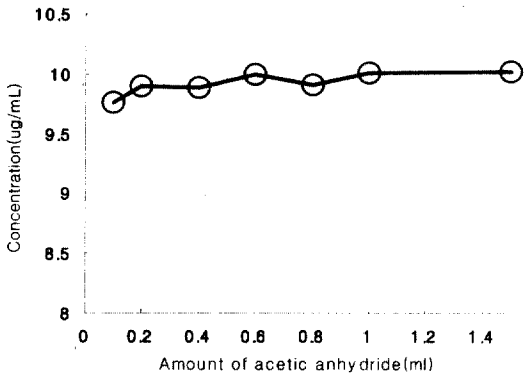

Fig. 1. Effect of acetic anhydride on the esterification of bisphenol-A(80℃, 10min).

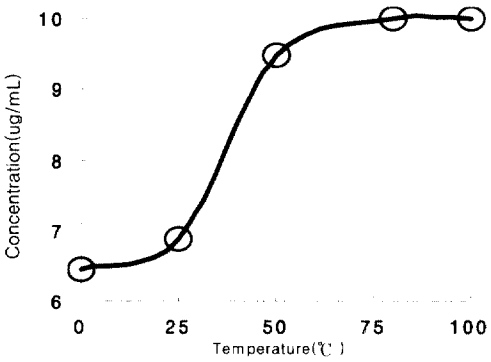

Fig. 2. Effect of temperature on the esterification of bisphenol-A (acetic anhydride 0.5 ml, 10 min).

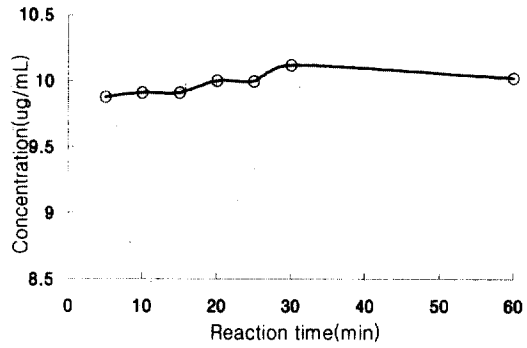

Fig. 3. Effect of reaction time on the esterification of bisphenol-A (acetic anhydride 0.5 ml, 80 ℃).

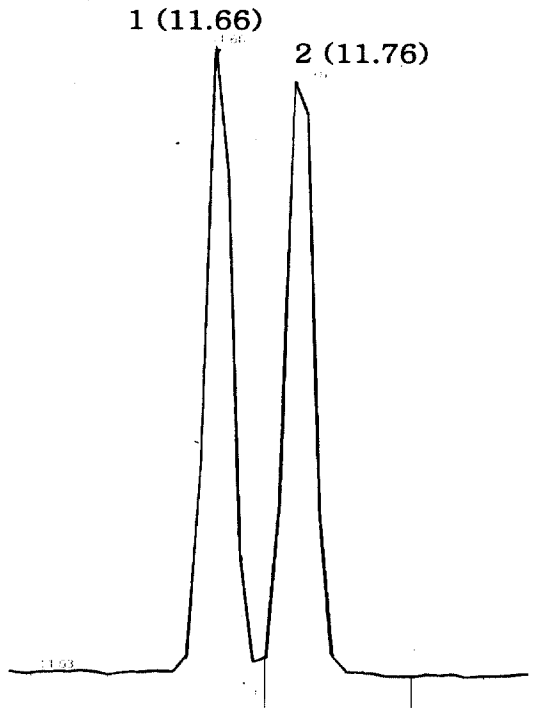

Fig. 4. GC/MS chromatogram of the derivatized bisphenol-A and the internal standard: 1=bisphenol-A derivative ; 2=bisphenol-A-d_{16} derivative.

Because two mole of acetic anhydride is needed to react with one mole of bisphenol-A (*Fig.* 4), 500 uL of acetic anhydride was enough to react with the trace bisphenol-A containing in the beverage.

The effect of the reaction temperature was performed at the temperature of 0, 25, 50, 80 and 100℃. The best conditions of the derivatization were obtained from at the

temperature of 80℃, and the reaction time was 10 min and the amount of acetic anhydride was 500 uL.(*Fig.* 2)

The effect of reaction time was studied for 0, 5, 10, 15, 20, 25, 30 and 60 min. The peak area was increased until 60 min and retained the constant area after 10 min. From these results, it can be optimized the factors influencing the reaction, such as 500 uL of acetic anhydride and the derivatizing time of 10 min at 80℃.(*Fig.* 3)

A typical chromatogram obtained with the above described conditions is shown in *Fig* 4. The analysis time was only within 15 min. The mass spectrum obtained from scan mode for this derivatized compound is shown in *Fig.* 5. The molecular ion appeared at m/z 312, while the base peak appeared at m/z 213.

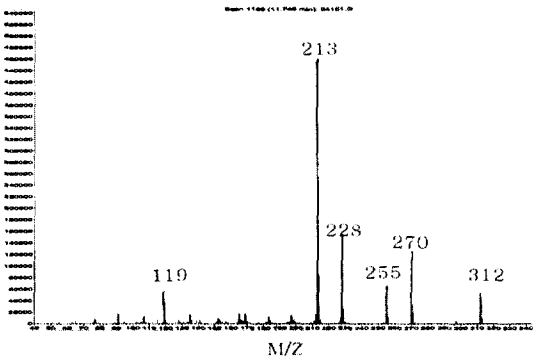

Fig. 5. Mass spectrum of the derivatized bisphenol-A.

For each standard chromatogram, the bisphenol-A to bisphenol-A-d_{16} area ratio was computed and plotted against the bisphenol-A in concentration of part per million (ppm). Calibration curve was linear in the range of 0.5 ~ 16 ng (*Fig.* 6). The each concentration of standard for the calibration curve was obtained from which the standard was spiked to 1 ml of acetone. And the concentration obtained from the calibration curve was converted to the original concentration (the original concentration will be 1/100). The correlation coefficient obtained for the calibration curve was 0.9994 and the equation for the calibration curve was y = 1.0391x - 0.1396. For the quantification of the bisphenol-A in the can material, the equations for the calibration curve was y = 1.0391 x - 0.1396. The coefficients of variation (C.V.) obtained from the repeated analysis of bisphenol-A of 0. 5~16 ng were within 0.4~3.0 %, both for the intra-day (n=3) and inter-day (n=3) experiments.

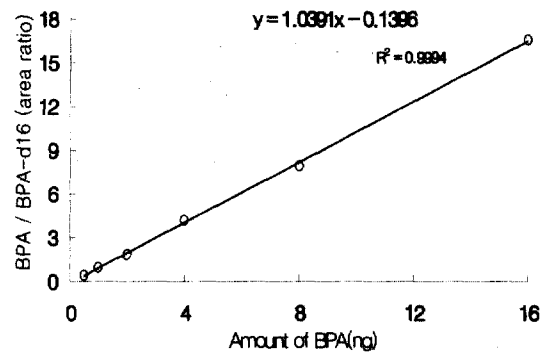

Fig. 6. The standard calibration curve of bisphenol-A obtained from the can material by GC/MS.

According to the method described above, some practical canned beverage samples were analyzed and the result is shown in *Table* 1. Bisphenol-A was detected the amount of 0.11~11.4 μg/can in drink cans. This developed method will be utilized to analyze the bisphenol-A from the various matrices.

Table 1. The amount of bisphenol-A in the canned beverages

Samples (No.)	Bisphenol-A concentration of can materials (μg/can)
Carbonated drink(7)	0.32 ~ 2.47
Coffee drink(4)	0.17 ~ 0.82
Beer(4)	0.58 ~ 1.16
Mixed drink(4)	0.29 ~ 11.40

References

1. I. B. Rufus, H. Shah and C. E. Hoyle, *J. Appl. Polym. Sci.*, **51**, 1549 (1994).
2. P. Paseiro, J. S. Lozano, S. Paz., P. Lopez and J. S. Gandara, *Anal. Chem.*, **345**, 527 (1993).
3. M. He, M. W. Urban and R. S. Bauer, *J. Appl. Polym. Sci.*, **49**, 345 (1993).
4. E. C. Dodds and W. Lawson. *Nature*, June 1936. p. 996.

5. A. V. Krishnan, P. Stathis, S. F. Per- muth, L. Tokes and D. Feldman, *Endocrinology*, **132,** 2279 (1993).
6. N. Olea, *Endocrinologta*, **41,** 39 (1994).
7. J. A. Brotons, M. F. Olea-Serrano, M. Villalobos, V. Pedraza and N. Olea, *Environmental Health Perspectives*, **103,** 608 (1995).
8. G. Challa and P. H. Hermans, *Anal. Chem.*, **32,** 778 (1960).
9. L. E. Brydia, *Anal. Chem.*, **40,** 2212 (1968).
10. M. L. Henriks-Eckerman and T. Lai- joki, *Analyst*, **113,** 239 (1988).
11. J. S. Gandara, S. P. Abuin, P. Paseiro and J. S. Lozano, *J. Chromatogr.*, **31,** 450 (1993).
12. R. J. Wingender, P. Niketas and C. K. Switala, *J. Coat. Tech.*, **70,** 75 (1998).