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# Bisphenol A – A problem for consumers of canned beer?

## Potential risks and analysis of debatable plastics used in canned beer coatings

**The precise determination of Bisphenol A (BPA) amounts in complex matrix like beer is presented. A practical, rapid and reliable sample preparation procedure for BPA determination is shown. The application of stable isotope dilution assay (SIDA) utilizing labmade deuterated BPA standards made it possible to obtain accurate analytical data. BPA concentrations in seven randomly chosen canned beers ranged from 1 µg BPA/L to 6.5 µg BPA/L. Compared to other canned foodstuff these amounts are low and presumably safe.**

Descriptors: Bisphenol A, stable isotope dilution assay, GC-MS, canned beer

## 1 Introduction

2,2-Bis-(4-hydroxyphenyl)-propane, commonly known as Bisphenol A or simply BPA, is a chemical agent existent in many products of everyday life. BPA is synthesized by coupling two equivalents phenol with acetone under hydrochloric acid catalysis. It is mainly employed as starting substance for the production of robust polycarbonate plastics (PC) and epoxy-phenolic resins, but also used in polyvinyl chloride (PVC). Industrial BPA production in EU countries accumulates to about one million tons per year [1].

PC plastics are prevalently utilized for the production of CDs, DVDs, mobile phones or computer cases due to their high resistance, ductility, and hardness. But, they are frequently used in food and beverage packaging, plastic table ware, or baby drinking bottles. Epoxy resins are part of paints, floor covers, adhesives as well as (water-)pipe linings. In food industry, reaction products of BPA with epichlorhydrin, forming Bisphenol A diglycidiyl ether (BADGE), are used. Those are predominantly introduced as inner can coatings in order to circumvent contact between can content and metal surface [2]. The presence of unpolymerized BPA is encountered the main problem when BPA polymers are used in immediate contact to food. Monomers originate from uncompleted polymerization, mechanical decomposition or cleavage by chemical hydrolysis. Those BPA monomers are reported to migrate from coating to packed product, being a source of contamination for foodstuff [3, 4, 5]. Consumption of products from BPA containing packages is considered the main source of consumers BPA exposure. Several research groups published BPA levels for various canned foods and beverages, however, data for BPA in beer is rare. In the literature analytical approaches differ considerably. They are mainly based on liquid chromatography coupled with mass spectrometry (LC-MS), a costly and uncommon technique in brewery quality assurance labs. A selection of BPA concentrations is illustrated in Table 1.

There are considerable differences in BPA concentrations between canned dairy products, fruits, vegetables, and soft drinks. Those are liable to characteristics of packed foodstuff (e.g. pH), different can types with varying thickness of resin coating as well as processing techniques. Especially the intensity of heat treatment is proposed to be highly significant. Whereas beverages usually are only subject to pasteurization (≥ 65 °C) canned food is exposed to higher temperatures (≥ 100 °C) [8].

The hygienic outcomes of oral BPA exposure today are still discussed very controversially. The major problem to evaluate BPA risks in humans is the lack of reliable data and long term studies. Animal tests show inconsistent results and do not adequately reflect the situation for humans. Especially, the action of BPA as endocrine disruptor does not allow direct comparison of animal testing data to humans. The nature of its estrogenic activity was already discovered in 1936 [9]. Later investigations, mainly applying rodents and aquatic organisms, showed BPA binds to and activates estrogenic receptors with multiple follow-ups for tested species [10]. In addition to the hormonal impact, BPA is reported as substrate of several metabolic reactions. It is converted into reactive intermediates and may also cause oxidative stress due to further degradation [11, 12, 13]. In contrast to multitude studies using various animal species, only limited data is available for the effects of BPA on humans. Nevertheless, a recently published study conducted on humans showed a correlation of high urinary BPA concentration and an increased risk for type II diabetes and cardiovascular disease [14]. Even critics note that in animal testing pure BPA is used and real uptake therefore is not correctly simulated, its impact on living organism cannot be denied. Renowned institutions engaged on this topic and guidelines concerning BPA exposure were established. The European Food Safety Authority

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Tables and figures see Appendix

(EFSA) proposed maximum amounts for BPA intake. At this the full Tolerable Daily Intake (TDI) was set to 50 µg BPA/kg bodyweight (bw), derived by applying a 100-fold uncertainty factor to the overall No-Observed-Adverse-Effect Level (NOAEL) of 5 mg BPA/kg bw/day. The EFSA also estimated the exposure to BPA by consumption of commercial foods and beverages to be 1.2 µg/kg bw/day. This calculation was based on the assumption a 60 kg adult consumes 1 kg of canned food (50 µg BPA/kg) and 2 liters of canned beverages (10 µg BPA/kg) per day [15]. In general, the EU enacted a legal ordinance obligating producers and importers of BPA to minimize risks for consumers and environment (REACH) [16]. Considering this, BPA limits are obsolete.

To gain deeper insights into the BPA complex of problems, the presented paper describes a gas chromatography-mass spectrometry (GC-MS) method for the precise determination of BPA amounts in complex foodstuff matrix such as beer. A practical, rapid and reliable sample preparation procedure for BPA determination in beer is presented. The application of stable isotope dilution assay (SIDA) utilizing labmade deuterated BPA ($^2H_4$-BPA or $D_4$-BPA) standards enabled obtainment of correct analytical data.

## 2 Material and Methods

### 2.1 Beer samples

Commercial beer cans (0,5 L contents) of seven breweries (here named A–G) were randomly chosen from convenience stores. By this, praxis conditions in age and stocking were simulated to guarantee proper sample conditions. Cans of three different manufacturers were used and samples size properly displays consumer's situation.

### 2.2 Stable Isotope Dilution Assay (SIDA) and standard synthesis

For BPA quantification beer samples were spiked with internal $D_4$-labelled BPA stable isotope standard. Standard synthesis was performed by autoclave treatment of unlabeled BPA (Sigma-Aldrich, purity ≥ 99 %) in deuterium oxide ($D_2O$, Sigma-Aldrich, purity 99.9 %). Temperature was set 160 °C for a 2-hour period (cf. Fig. 2). Crude $D_4$-BPA was purified by flash chromatography, identity as well as labeling degree was confirmed by gas chromatography-mass spectrometry (GC-MS).

### 2.3 Sample extraction, solid phase extraction (SPE) and derivatization

Canned beers were casted into 1 L glassware. $D_4$-BPA was added to adjust a standard concentration of 5 µg/L and degassing was performed (10 minutes in ice chilled ultrasonic bath). For deprotonation of BPAs hydroxyl-functions sample pH was adjusted to 8.5 by dropwise addition of 0.1 N ammonia solution. This was essential for solid phase extraction (SPE) of BPA. At first, aminopropyl-modified silica phases (Macherey-Nagel, Chromabond® $NH_2$, 1000 mg), afterwards RP-18E phases (Merck, LiChrolut® RP-18E, 500 mg) were used for analyte enrichment and effective matrix removal. Both phases were conditioned before charging (aminopropyl with each 4 mL of 0.1 N ammonia solution and water, RP18E with each 2 mL methanol and water). Subsequently, 100 mL sample was subjected to Cromabond®-$NH_2$-phases. Matrix components were eluted by washing with water and 0.1 N ammonia solution (each 2 mL). SPE was dried under vacuum (200 mbar) and BPA was eluted with 4 mL 0.01 N hydrochloric acid, directly applying eluate onto preconditioned RP-18E. The analyte was obtained by washing SPE with water (2 mL), vacuum drying and elution with methanol (2 mL). Methanol eluate was concentrated to dryness. Residue was either simply dissolved in pentane or further derivatized. For silyl- derivate formation each 50 µL of pyridine (Sigma-Aldrich, purity ≥ 99.8 %) and N,O-Bis-(trimethylsilyl)trifluoroacetamide (BSTFA, Sigma-Aldrich, purity ≥ 99 %) were added and heated to 80 °C for 60 minutes. Work up was performed in triplicate for each beer sample.

### 2.4 GC-MS analysis

GC-MS analysis was performed on Shimadzu GCMS-QP2010 Plus applying RXI® 5 ms column (Restek, inner Ø = 0.25 mm, length = 30 m) for GC separation. Helium was used as carrier gas. Injector temperature was set to 240 °C, head pressure to 76 kPa. Splitless injection was performed with 0.8 minutes sampling time and an injected volume of 2 µL. Column oven temperature program was set at an initial temperature of 70 °C for 80 seconds, raised by a rate of 8 °C/minute until reaching 230 °C, then 50 °C/minute up to 300 °C. For column flushing again a holding time of seven minutes was maintained. In sum total program lasted for 29.56 minutes. Ion source of quadrupole mass spectrometer (QP-MS) was adjusted to 200 °C, interface temperature to 250 °C. Analyte ionization was reached by electron impact ionization (EI), selected ion monitoring (SIM) was used to increase sensitivity and intensity.

### 2.5 Calibration

For calibration $D_4$-BPA standard was adjusted to a concentration of 10 µg/L, commercial BPA (Sigma-Aldrich, purity ≥ 99 %)) was diluted to concentrations of 0, 5, 10, 25, 50 and 100 µg/L using methanol as solvent. Solutions were equally mixed (1:1) and concentrated to dryness. Dry residue was either directly dissolved in 1000 µL of diethyl ether (VWR, water < 0.01 %) or derivatized. For derivatization each 50 µL of pyridine and BSTFA were added (80 °C/60 minutes). Derivatized mixtures were dissolved in diethyl ether before injection. GC-MS setup was applied as described above, repeated determination was performed as a matter of course. Area ratios ($D_4$-BPA / BPA) were plotted against concentration ratios ([µg $D_4$-BPA/L] / [µg BPA/L]) of standard and analyte. For TMS derivates line of best fit ($y = 0.056x + 0.008$) and stability index ($R^2 = 0.999$) were calculated using Microsoft Excel®. Calibration without derivatization showed similar results.

### 2.6 Quantification

The identification of BPA and $D_4$-BPA standard was based on retention time as well as on ion ratios. Retention time of both, analyte and standard, were identical within the range of 0.02 s (cf. Fig. 3). Quantification was performed by selected ion monitoring mass spectrometry (SIM-MS) mode. BPA exhibited ions at m/z = 213 and D$_4$-BPA at m/z = 217 (213+4). TMS derivatized BPA exhibited prominent ions at 357 and 361, respectively. Integration of the respective SIM-MS peak areas enabled precise calculation of BPA amounts.

## 3 Results and Discussion

### 3.1 Results of BPA identification and quantification by stable isotope dilution assay (SIDA)

Quantification of BPA via SIDA was carried out by addition of D$_4$-BPA standard to beers at the very beginning of sample preparation. Therefore, sample loss during workup is considered by internal standard and associated errors are eliminated [17]. Isotope standard bear the same physical and chemical properties e.g. boiling point, solubility, acidity, reactivity, and their GC characteristics are also very similar. Consequently both substances, analyte and standard, could be identified by means of their retention times (11.7 minutes). Quantification can be conducted by means of peak area integration of SIM-MS ion traces because EI molecular- and fragment ions of BPA and D$_4$-BPA show same intensities. Figure 3 shows SIM-MS (m/z = 213 and m/z = 217) chromatograms for BPA and D$_4$-BPA. Bis-O-trimethylsilyl- (-Si(CH$_3$)$_3$) derivates of BPA and D$_4$-BPA exhibited SIM-MS ions at m/z = 357 and 361, respectively. The derivates have longer retention times at same GC conditions (20.5 minutes) and their GC peak profile might be better especially when column performance is low.

Figure 3 shows the SIM chromatograms (m/z 213 (BPA) and m/z 217 (D$_4$-BPA) and demonstrates the fact D$_4$-BPA exhibits only a 0.02 s shift in retention time compared to BPA. Compared to commercially available D$_6$- and D$_{16}$-BPA standards, this property of D$_4$-BPA is a major advantage. A decreased retention time of deuterated compounds is commonly observed even their mass is higher. This shift in retention time was especially observed for D$_{16}$-BPA. Changes in retention time of standard and analyte might lead to misinterpretations (cf. *Ackerman et al.* [18]).

As stated above one severe advantage of isotope standards is their identical physical and chemical behavior during sample work up. A second advantage is their behavior at electron impact (EI)-MS conditions. Here, intensities of hydrogen and deuterium containing fragment ions can directly be compared and used for accurate quantification. Figure 4 illustrates mass spectra of a BPA and D$_4$-BPA mixture. Their molecular mass difference of four units is explicitly visible for molecular ion peak (BPA m/z = 228 and D$_4$-BPA m/z = 232) as well as for basepeak (BPA m/z = 213 and D$_4$-BPA m/z = 217, $[M^{+\circ}-CH_3^{\circ}]$). EI-MS fragmentation reactions lead to m/z = 119 and 91 (BPA) and m/z = 121 and 93 (D$_4$-BPA) by scission of phenolic bonds whereby two deuterium atoms are lost. This fragmentation reaction seems to be weakly influenced by the H- to D- exchange. Therefore, m/z = 213 and 217 were used for quantification.

### 3.2 Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) were determined by signal to noise ratio (S/N). S/N > 9 is defined as limit of quantification (LOQ), S/N > 3 is defined as limit of detection (LOD). LOQ for BPA was 0.1 µg/L, LOD 0.04 µg/L.

### 3.3 Results of beer analysis

The established method was used to determine canned beer BPA levels. The complex beer matrix required an extensive but "easy to handle" sample preparation via NH$_2$- and RP18E SPE. Data for canned beers are given as mean values of BPA concentrations. Samples were prepared and measured in triplicate, error bars are included (Fig. 5).

Maximum BPA levels were detected in canned beer of brewery A (6.6 µg BPA/L), lowest concentration of 1.9 µg BPA/L in cans of brewery E. Mean value of all tested beers was 4.2 µg BPA/L. However, much higher levels were found in canned fruits and vegetables, BPA levels determined in beer are in the same range as those of other beverages (cf. Table 1). Matching concentrations may accord to comparable product pH, carbonation, packaging and processing properties. A major difference between food and beverages may be caused by heat treatment. Beverages usually are subject to pasteurization (≥ 65 °C), whereas canned food is exposed higher temperatures (≥ 100 °C).

## 4 Conclusion and outlook

The present paper describes a simple, fast and cost-effective work-up procedure and determination method for BPA in beer samples. By dual SPE extraction analyte enrichment was reached and complex beer matrix could be removed to a great extend. Utilization of stable isotope dilution assay (SIDA) enabled a highly sensitive GC-EI-SIM-MS analysis of BPA in beer. By use of labmade D$_4$-labelled stable isotope standard direct and accurate quantification of BPA was achieved. BPA concentrations in seven randomly chosen canned beers ranged from 1µg BPA/L to 6.5 µg BPA/L. Compared to other canned foodstuff these amounts are low and presumably safe. BPA uptake for a 70 kg adult consuming 1 L of canned beer containing 6.5 µg BPA/L (maximum ascertained value) per day is 0.09 µg/kg day. Drawn to the TDI of 50 µg /kg day this accounts to be less than 0.2 %. In addition BPA concentrations are below the limit value proposed for beverages by EFSA [15]. However, BPA is a crucial topic. It was demonstrated as endocrine disruptor and is currently widespread in nature. Further restrictions and regulations for manufacturers, importers and users (such as breweries) of BPA where announced by UBA [1]. In the future, one cannot exclude BPA is completely banned for any applications like in food packaging. For all these reasons a frequent analytical monitoring of foods and beverages may be very useful to identify changes and to inform the consumer.

## 5 References

1. Umweltbundesamt: Bisphenol A – Massenchemikalie mit unerwünschten Nebenwirkungen Aktualisierte Fassung Juli 2010.
2. Cao,X.L.; Corriveau,J. and Popovic, S.: Levels of bisphenol A in canned soft drink products in canadian markets, J. Agric. Food Chem., **57** (2009), pp. 1307-1311.

3. Feldman, D. and Krishnan, A.: Estrogens in unexpected places: possible implications for researchers and consumers, EnViron.Health. Perspect., **103** (1995), pp. 129-133.
4. Goodson, A.; Summerfield, W. and Cooper, I.: Survey of bisphenol A and bisphenol F in canned foods, Food Addit. Contam. **19** (2002), pp. 796-802.
5. Biles, J. E.; McNeal, T. P and Begley, T. H.: Determination of Bisphenol A migrating from epoxy can coatings to infant formula liquid concentrates, J. Agric. Food Chem., **45** (1997), pp. 4697-4700.
6. Braunrath, R.; Podlipna, D.; Padlesak, S. and Cichna-Markl, M.: Determination of bisphenol a in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection, J. Agric. Food Chem. **53** (2005) , pp. 8911-8917.
7. Kang, J. H. and Kondo, F.: Bisphenol A migration from cans containing coffee and caffeine. Food Addit. Contam., **19** (2002), pp. 886-890.
8. Munguia-Lopez, E. and Soto-Valdez, H.: Effect of heat processing and storage time on migration of bisphenol A (BPA) and bisphenol A-diglycidyl ether (BADGE) to aqueous food stimulant from Mexican can coatings, J. Agric. Food Chem., **49** (2001), pp. 3666-3671.
9. Dodds E.C. and Lawson W.: Synthetic estrogenic agents without the phenanthrene nucleus. Nature **137** (1936), p. 996.
10. National Center for Environmental Assessment Office of Research and Development U.S. Environmental Protection Agency, A cross-species mode of action information assessment: A case study of Bisphenol A. Report EPA/600/R-05/044F.
11. Bindhumol, V.; Chitra, K. C. and Mathur, P.: Bisphenol A induces reactive oxygen species generation in the liver of male rats, Toxicology, **188** (2003), pp. 117-124.
12. Atkinson, A. and Roy, D.: In vivo DNA adduct formation by bisphenol A, EnViron. Mol. Mutagen., **26** (1995), pp. 60-66.
13. Wetherill, Y.; Akingbemi, B.; Kanno, J.; McLachlan, J.;. Nadal, A. and Sonnenschein, C.: In vitro molecular mechanisms of bisphenol A action, Reproductive Toxicology 2007 (24), pp. 178-198.
14. Lang, I.; Galloway, T.; Scarlett, A.; Henley, W.; Depledge, M.; Wallace, R. and Melzer,D.: Association of urinary Bisphenol A concentration with medical disorders and laboratory abnormalities in adults, JAMA September 2008; 300, pp. 1303-1310.
15. European Food Safety Authority: Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-Bis(4-Hydroxyphenyl)propane (Bisphenol A), The EFSA Journal (2006) 428.
16. Europäische Union. (2007), Verordnung (EG) Nr. 1907/2006 des europäischen Parlamentes und des Rates vom 18. Dezember 2006, berichtigte Fassung vom 29. Mai 2007 zur Registrierung, Bewertung, Zulassung und Beschränkung chemischer Stoffe (REACH).
17. Hübke, H.; Garbe,L.-A. and Tressl.,R.: Characterization and quantification of free and esterified 9- and 13-Hydroxyoctadecadienoic Acids (HODE) in barley, germinating barley, and finished Malt, J. Agric. Food Chem., **53** (2005), pp 1556-1562.
18. Ackerman L.; Noonan G.; Heiserman, W.; Roach. J.; Limm, W. and Begley, T.: Determination of bisphenol A in U.S. infant formulas: Updated methods and concentrations, J. Agric. Food Chem., **58** (2010), pp. 2307-13.

*Received 30 August, 2010, accepted 07 October, 2010*

# Appendix

**Table 1 Concentration of BPA in different canned foodstuffs**

| Type of canned foodstuff | BPA [µg/L] or [µg/kg] | Reference |
|---|---|---|
| Vegetables | 23.9 | [6] |
| Fruits | 10.5 | [6] |
| Dairy products | 31.0 | [7] |
| Soft drinks | 1–4.5 | [2] |

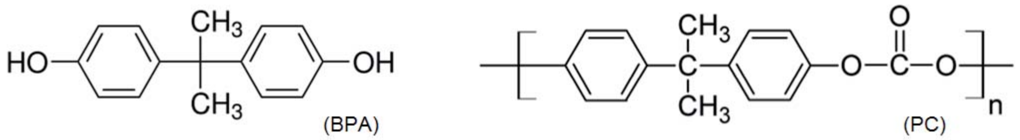

**Fig. 1 Original Bisphenol A molecule and as a monomer unit of polycarbonate plastics (PC)**

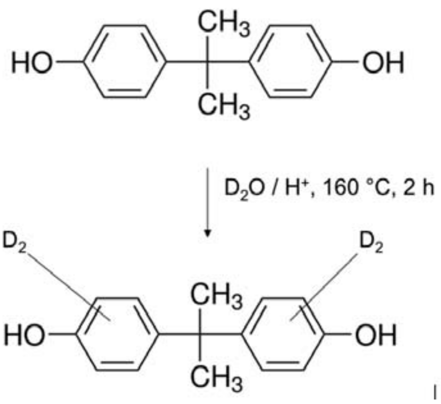

**Fig. 2** Synthesis of $D_4$-stable isotope standard by autoclaving of BPA in $D_2O$

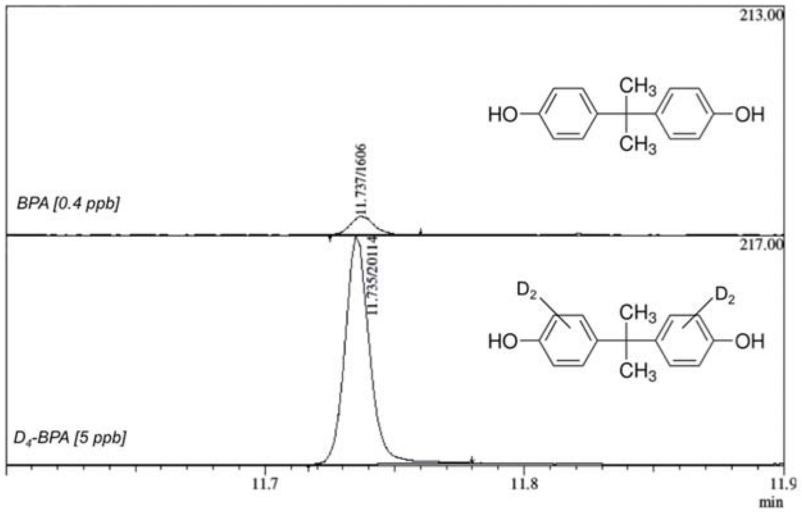

**Fig. 3** SIM-MS chromatograms (m/z = 213 and 217) of BPA and $D_4$-BPA in comparison

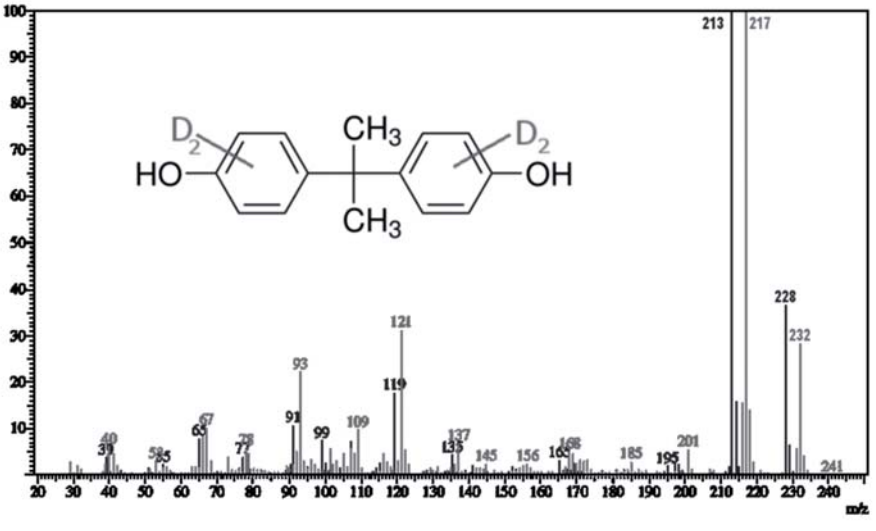

**Fig. 4** Matching EI-MS spectra of BPA and $D_4$-BPA

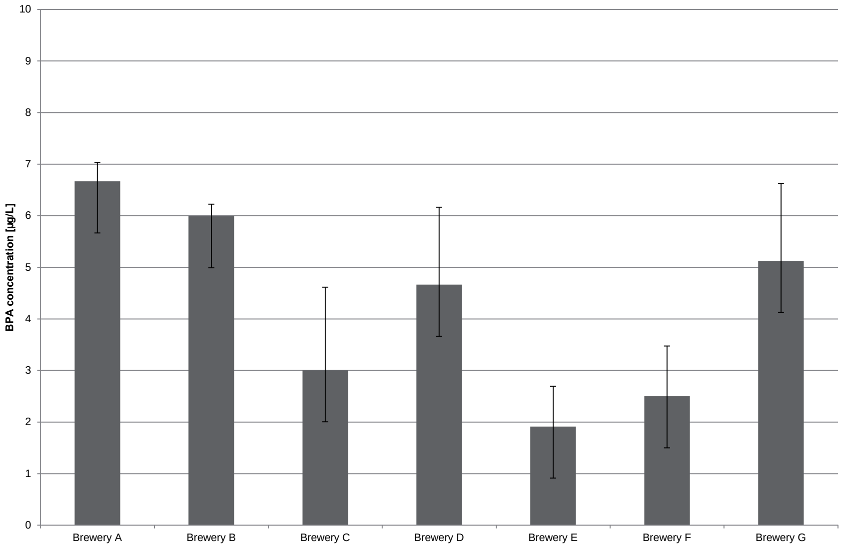

**Fig. 5** Comparison of BPA concentration ascertained in canned beers of seven randomly selected breweries