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Zdenka Brenn-Struckhova, Margit Cichna-Markl

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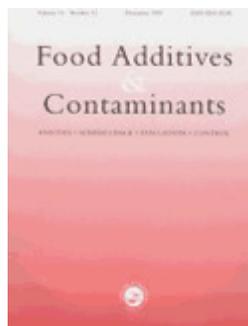
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Determination of bisphenol A in wine by sol-gel immunoaffinity chromatography, HPLC and fluorescence detection

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3 **Determination of bisphenol A in wine by sol-gel immunoaffinity**
4 **chromatography, HPLC and fluorescence detection**
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10 **Z. Brenn-Struckhova and M. Cichna-Markl**
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12
13
14 Department of Analytical and Food Chemistry, University of Vienna,
15 Währinger Straße 38, A-1090 Vienna, Austria
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24
25 Correspondence to: Dr. Margit Cichna-Markl
26 Institut für Analytische Chemie und Lebensmittelchemie
27 Universität Wien
28 Währinger Straße 38
29 A-1090 Wien, Austria
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35 E-mail address: margit.cichna@univie.ac.at
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Abstract:

The paper presents a highly selective analytical method for the determination of traces of bisphenol A (BPA) in wine and the results of a survey 59 wine samples sourced from vats (steel, wood and plastic), glass bottles and Tetra Briks. The procedure consists of sample clean-up by sol-gel immunoaffinity chromatography followed by determination of BPA by high performance liquid chromatography and fluorescence detection. The method has a LOD (S/N=3) of 0.1 ng/ml and a LOQ (S/N=6) of 0.2 ng/ml. In 13 of 59 wine samples the BPA concentration was below the LOQ. The mean and median for all wine samples with BPA concentrations above the LOQ were 0.58 and 0.40 ng/ml, respectively. These values – the first set of data on BPA in wine - are far lower than previously published BPA levels derived from migration experiments using wine simulants. Experiments carried out by submerging plastic stoppers in ethanol-water (11:89, v/v) up to 11 weeks indicated that detectable amounts of BPA can be leached from some stoppers.

Key words:

Bisphenol A, wine, migration, immunoaffinity chromatography, sol-gel, HPLC

Introduction

Bisphenol A (BPA) is an industrial chemical primarily used in the production of polycarbonates and epoxy resins. Many food containers - like returnable milk and water bottles or baby milk bottles - are made of polycarbonate, while epoxy resins are used as protective coatings in metal food and beverage cans or to line wine storage vats. A number of studies showed a migration of BPA traces from polycarbonate based packagings (Biles *et al.* 1997a, Brede *et al.* 2003, Nerin *et al.* 2003), can coatings (Munguia-Lopez and Soto-Valdez 2001, Goodson *et al.* 2004) or wine vat coatings (Larroque *et al.* 1989) into food or wine simulants.

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3 BPA belongs to the so-called endocrine-disrupting chemicals (EDCs) which can
4 interfere with the function of the hormonal system of humans and animals by
5 binding to the natural estrogen receptor. In animal experiments BPA has been
6 shown to influence mammary gland development (Markey *et al.* 2001) and cause
7 urethral malformations (Timms *et al.* 2005) or meiotic aneuploidy (Hunt *et al.*
8 2003). Recent studies indicated that BPA can be biologically active at very low
9 doses which had not caused detectable effects in toxicological studies and had
10 therefore been assumed to be safe for humans (Timms *et al.* 2005, Ashby 2003,
11 Welshons 2003).
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21 Since the food chain is considered as the main source of BPA exposure for
22 humans, several surveys have already been carried out to determine BPA levels
23 in canned foodstuffs and beverages (Biles *et al.* 1997b, Yoshida *et al.* 2001,
24 Goodson *et al.* 2002, Brotons *et al.* 1995, Thomson and Grounds 2005,
25 Braunrath *et al.* 2005) but there is a lack of realistic data for wines. Both the
26 “Opinion of the Scientific Committee on Food on BPA” (SCF 2002) and
27 the “European Union risk assessment report on BPA” (EURAR 2003) were based
28 on exposure models derived from data published by Larroque *et al.* (1989) which
29 were, however, obtained in experiments with wine simulants. Following the
30 “Opinion of the Scientific Committee on Food on BPA” the European Commission
31 reduced the Specific Migration Limit for BPA from 3 mg/kg to 0.6 mg/kg (EC
32 2004).
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44 A recent paper by Lambert and Larroque (1997) advanced the methodology for
45 the determination of BPA (and other phenolic compounds) in wine which had
46 been in contact with epoxy resins by HPLC and fluorescence detection but due to
47 its high detection limit the method could only be validated by using fortified
48 samples.
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55 The present paper describes a method for the determination of BPA in wine
56 which includes a slightly modified sample clean-up procedure previously
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3 developed for the determination of BPA in canned beverages, fruits and
4 vegetables (Braunrath and Cichna 2005). The method offers a detection limit low
5 enough to finally allow the analysis of real wine samples. The application of the
6 method described in this paper provides the first set of data on BPA in wine
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12 13 14 **Experimental**

15 16 17 *Reagents and Chemicals*

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21 Purified polyclonal bisphenol A (BPA) rabbit antibodies (5 mg/240 µl PBS buffer)
22 were a gift from Japan EnviroChemicals, Ltd. (Tokyo, Japan). BPA was bought
23 from Sigma (St. Louis, MO, USA), tetramethoxysilane (TMOS) from Fluka
24 (Buchs, Switzerland). Acetonitrile (ACN), gradient grade for HPLC, was delivered
25 from Fisher Scientific (Leicestershire, UK). The BPA stock solution was prepared
26 by dissolving 20.0 mg of BPA in 20.0 ml ACN. Lower concentrated BPA solutions
27 were obtained by diluting the stock solution with mobile phase. All BPA solutions
28 were stored at 4 °C. Phosphate-buffered saline (PBS), pH 7.6, was prepared by
29 dissolving 12.46 g Na₂HPO₄ × 2 H₂O, 1.56 g NaH₂PO₄ × 2 H₂O and 8.5 g NaCl in
30 1 litre of bidistilled water.
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40 41 *Preparation of Sol-Gel Columns*

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44 Sol-gel immunoaffinity columns were prepared according to a previously
45 described protocol (Braunrath and Cichna 2005). In brief, TMOS was hydrolysed
46 under slightly acidic conditions in an ultrasonic bath for 30 min. After mixing 1 ml
47 hydrolysate with 1 ml of PBS containing 1 mg of BPA antibody a gel was formed
48 within a few min. When the resulting sol-gel glass had lost 50% of its initial mass
49 it was manually ground in an achate mortar and packed into a 3 ml glass column
50 (Merck, Darmstadt, Germany) equipped with a polytetrafluorethylene frit (Merck).
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3 After flushing the immunoaffinity column in sequence with 20 ml of PBS, 15 ml of
4 ACN-water (40:60, v/v) and 20 ml of PBS, it was stored in PBS at 4 °C.
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8 Sol-gel filter columns were prepared following the same procedure but by mixing
9 PBS (without BPA antibodies) with pre-hydrolysed TMOS.
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12 13 *Wine samples*

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17 59 wine samples were analysed in the present study. 31 samples were obtained
18 from four Austrian vintagers from the Wachau, a famous Austrian wine-growing
19 region. Vintagers kindly provided information about wine storing conditions, e.g.
20 the tank material and storage time. Ten samples were taken directly from vats,
21 21 had already been filled into glass bottles. Twenty-eight wine samples (17 filled
22 into glass bottles and 11 Tetra Brik wines) were bought in local markets. Since
23 white wine production is more important in Austria (especially in the Wachau)
24 than red wine production the study comprised more white wine (46) than red
25 wine (13) samples.
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35 *Sample Preparation*

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38 The sample pretreatment procedure is a slight modification of the procedure
39 developed for the analysis of canned beverages, fruits and vegetables
40 (Braunrath and Cichna 2005) which was now adapted to the analysis of wine. It
41 is very simple and consists of just a few steps.
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48 Wine samples were degassed in an ultrasonic bath for 5 min before taking an
49 aliquot of 25 ml which was mixed with 25 ml of PBS. The pH values of these
50 samples were in the range from 3.0 to 3.9. In order to enable interactions of BPA
51 and the antibodies immobilised in the immunoaffinity column the pH value of the
52 sample was adjusted to pH 7.0 by adding diluted sodium hydroxide. To avoid
53 clogging of the immunoaffinity column the samples were filtered through a
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3 borosilicate frit. The sample was then applied to a sol-gel pre-column which was
4 on-line coupled with an immunoaffinity column using a flow-rate of about 1
5 ml/min. After sample application the immunoaffinity column was decoupled from
6 the pre-column and washed with 5 ml of ACN-water (10:90, v/v) before eluting
7 BPA with 4 ml of ACN-water (40:60, v/v). The eluate was collected in a 5 ml
8 measuring flask and filled up to the ring mark with bidistilled water. An aliquot of
9 100 µl was injected into the HPLC system. The immunoaffinity column was
10 regenerated with 20 ml of PBS.
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19 *HPLC separation and detection*

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23 In the present work two HPLC systems were used operated with different phase
24 systems and detectors.
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28 HPLC system 1, used to determine BPA concentrations in wine samples,
29 consisted of a high pressure gradient pump (Model L-6200, Merck), a column
30 thermostat (Column oven 655 A-52, Merck), a six-port injection valve (Model
31 7161, Rheodyne) equipped with a 100 µl injection loop and a fluorescence
32 detector (Model 1080, Merck) set at 275/305 nm. Wine samples were analysed
33 using a LiChrospher 60 RP-Select B column, 5 µm, 250 x 4.0 mm I.D. (Merck).
34 The mobile phase consisted of water-ACN (70:30, v/v). The flow rate was 1.0
35 ml/min. Peaks were integrated using the Stratos V 3.0 software (Polymer
36 Laboratories, Darmstadt, Germany). At the end of each HPLC analysis the
37 analytical column was flushed with water/ACN (40:60, v/v) for 10 min.
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48 HPLC system 2 was used in order to confirm the identity of BPA when migration
49 experiments with plastic stoppers were carried out. It consisted of a high
50 pressure gradient pump (Model L-6200, Merck), an autosampler (Model AS-2000
51 A) and a coulometric electrode array detector (ESA, Chelmsford, MA, USA)
52 equipped with two cell blocks consisting of eight working electrodes. The
53 analytical column was a HP-Hypersil PDS column, 5 µm, 250 x 4 mm I.D. Mobile
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3 phase A was 0.01 M sodium acetate buffer (pH 4.8, adjusted with acetic acid),
4 mobile phase B ACN. The gradient program was as follows: 0 min: 77% A / 23%
5 B, 10 min: 76.1% A / 23.9% B, 20 min: 73.6% A / 26.4% B, 30 min: 66.8% A /
6 33.2% B, 40 min: 60% A / 40% B, 62 min: 60% A / 40% B. The flow-rate was 0.6
7 ml/min, the injection volume 100 µl. Potentials were set at +300, +400, +500,
8 +550, +600, +650 and +700 mV against palladium reference electrodes, the
9 eighth electrode did not work. Peaks were integrated using the CoulArray Win
10 software.
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19 *External calibration*

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22 HPLC system 1 was calibrated by injecting nine BPA standard solutions in the
23 concentration range from 0.3 to 100.0 ng/ml in mobile phase. Analysis functions
24 were obtained by linear regression of peak areas on standard concentrations.
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29 *Standard addition method*

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32 In order to determine BPA recoveries the standard addition method was carried
33 out with one white wine (sample 33) and one red wine (sample 55). The non-
34 spiked and three spiked samples (+ 100, 200 and 300 µl of a 100.0 ng/ml BPA
35 standard / 25 ml wine sample) were analysed as described above. Peak areas
36 were plotted against the amount of analyte added. Recoveries were determined
37 by dividing the slope of the linear regression line for the standard addition by the
38 slope of the linear regression line of BPA standard solutions.
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48 *Solid phase extraction*

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51 Solid phase extraction experiments were carried out using 3 ml C₁₈ cartridges
52 packed with 500 mg Isolute (International Sorbent Technology, Mid Glamorgan,
53 UK). The C₁₈ column was flushed with 6 ml of ACN, followed by 6 ml of ACN-
54 water (10:90, v/v). Wine samples were applied at a flow-rate of about 1 ml/min.
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3 After washing the column with 10 ml of ACN-water (20:80, v/v) elution was
4 carried out with 4 ml of ACN, collecting the eluate in a 5 ml measuring flask. The
5 eluate was evaporated under a gentle nitrogen stream to about 2 ml before filling
6 up the flask with bidistilled water to the ring mark.
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10 11 12 *Migration of BPA from plastic stoppers*

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15 Each of four plastic stoppers from different manufacturers was stored submerged
16 in a 50 ml centrifuge tube filled with 10 ml of ethanol-water (11:89, v/v). Since in
17 most wine samples the alcohol concentration was in the range from 11 to 12%,
18 migration experiments were carried out with 11% ethanol instead of 10% ethanol
19 conventionally used as alcoholic simulant. At the end of the storage period a 100
20 μ l aliquot of the ethanolic solution was directly injected into HPLC system 1.
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28 **Results and discussion**

29 30 31 32 *Sample clean-up*

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35 Fig. 1 shows typical chromatograms obtained by injecting (A) a BPA standard
36 solution, (B) a white wine and (C) a red wine sample, both purified by
37 immunoaffinity chromatography. In order to illustrate the increased efficiency in
38 removing matrix components obtained by the immunoaffinity column the same
39 wine samples were alternatively purified by solid-phase extraction using phase
40 systems and operating procedures (as described in the experimental section)
41 which had been optimized for maximum BPA recovery (Braunrath and Cichna
42 2005). The chromatograms obtained are documented in Fig. 2.
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50 [Insert Figure 1 and Figure 2 about here]

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53 Comparing Fig. 1 (B) and Fig. 1 (C) with Fig. 2 clearly demonstrates the
54 significantly lower efficiency of the SPE cartridges in removing matrix
55 components (Please note the different scaling of the y-axis in Fig. 1 and Fig. 2).
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Method performance characteristics

HPLC system 1 was calibrated by injecting 100 µl of BPA standard solutions from 0.3 to 100.0 ng/ml, obtaining a good linearity ($r > 0.9998$, $n=9$) between BPA concentration and peak area. The limit of detection (LOD), calculated for a signal-to-noise ratio of 3, was 0.5 ng/ml.

The analytical performance of the whole analysis method was assessed by applying the standard addition method. Correlation coefficients, mean recoveries, standard deviations of the recovery and the LODs ($S/N=3$) and LOQs ($S/N=6$) are given in Table 1.

[Insert Table 1 about here]

Since the sample preparation procedure results in an analyte enrichment factor of five the whole analysis method offers a LOD and LOQ of 0.1 and 0.2 ng/ml, respectively.

BPA levels in wine samples

In order to get a first impression in the BPA concentrations found in commercially available wine, 59 wine samples were collected, analysed and the results listed together with information on factors which might influence the BPA concentrations. The data obtained are shown in Table 2 and Table 3.

[Insert Table 2 and 3 about here]

In two of ten samples taken directly from wine vats BPA concentrations were below the LOQ (see Table 2a). In seven samples BPA levels ranged from 0.2 to 0.5 ng/ml; only in one sample a significantly higher BPA level of 2.1 ng/ml was found. Mean and median were 0.50 ng/ml and 0.20 ng/ml, respectively. In six of

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3 21 wine samples which were obtained from local vintagers but had already been
4 filled into bottles the BPA concentrations were below the LOQ. In 15 wine
5 samples BPA concentrations ranged from 0.2 ng/ml to 1.5 ng/ml, with a mean of
6 0.61 ng/ml and a median of 0.50 ng/ml.
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12 In five of the 28 wine samples purchased at local markets the BPA concentration
13 was below the LOQ. In 23 samples BPA levels were in the range from 0.2 to 1.6
14 ng/ml. The mean was 0.58 ng/ml, the median 0.40 ng/ml.
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19 In general, BPA concentrations were very low. The mean and median for all wine
20 samples with BPA concentrations above the LOQ were 0.58 and 0.40 ng/ml,
21 respectively. In all samples BPA concentrations were far below the Specific
22 Migration Level of 0.6 mg/kg established by the EU Commission in 2004.
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28 The data obtained indicate that neither the material of the vat nor the storage
29 time of the wine has an influence on the BPA concentration in the wine sample.
30 Furthermore, no significant difference has been found between the BPA levels in
31 white wine or red wine samples. In addition there was no relation between the
32 alcohol concentration and the BPA level of the sample. Neither the packaging
33 material (glass bottle or Tetra brik) nor the top of the glass bottle (plastic, metal
34 or cork) had a measurable effect on the BPA level.
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42 *Migration of BPA from plastic stoppers.*

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46 In order to investigate if BPA can migrate from a plastic stopper four different
47 plastic stoppers were stored in 50 ml centrifuge tubes submerged in 10 ml of
48 ethanol-water (11:89, v/v). Table 4 gives the BPA amounts determined in the
49 ethanolic solutions after storage periods from five to 11 weeks by injecting
50 aliquots of the incubation solutions into HPLC system 1. Figure 3 shows the
51 chromatogram of the ethanolic solution after incubation of stopper 4 for 11
52 weeks.
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5 [Insert Figure 3 and Table 4 about here]
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9 From two stoppers detectable amounts of BPA were released into the ethanolic
10 solution. In order to confirm the identity of BPA one incubation solution was also
11 injected into HPLC system 2 which was equipped with a coulometric electrode
12 array detector. Figure 4 shows the chromatograms of (A) a BPA standard
13 solution and (B) the incubation solution. Both chromatograms contained a peak
14 with a retention time of 48 min, indicating the presence of BPA in the ethanolic
15 solution. Figure 5 demonstrates the similarity of the current-voltage curves of the
16 48 min peaks, adding a further evidence for the identity of BPA peaks in the
17 chromatogram of the ethanolic solution and thus migration of BPA from the
18 plastic stopper.
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21 [Insert Figure 4 and Figure 5 about here]
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29 However, migration of BPA from plastic stoppers will only lead to insignificant
30 contributions to the BPA concentrations. (The migration of 30 ng BPA from a
31 plastic stopper into a wine sample contained in a 750 ml glass bottle would result
32 in a concentration of about 40 pg BPA / ml wine which is far below the Specific
33 Migration Level of 0.6 mg/kg established by the EU Commission).
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39 40 *BPA exposure due to wine consumption* 41

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44 Due to the lack of literature data on BPA concentrations in wine the results
45 obtained in the present study cannot be compared with previously published
46 ones. The data can, however, be used to reconsider the risk assessment
47 obtained with the help of exposure models based on the results of experiments
48 with wine simulants by Larroque et al. (1989). In their study three series of
49 migration experiments were carried out by placing into contact wine simulants
50 with inert materials coated with resins. From the highest BPA migration level
51 obtained (100 mg BPA/kg resin in series 1) the worst-case BPA concentration in
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3 wine was calculated to be 650 ng g^{-1} , assuming that the wine has been stored in
4 a 1500 L vat lined with 10 kg resin. The BPA concentrations obtained in the
5 present paper were far lower than this worst-case estimate. BPA risk assessment
6 by the Scientific Committee on Food on BPA was also based on the migration
7 study of Larroque et al. (1989). However, the SCF calculated a significantly
8 lower BPA concentration of 9 ng g^{-1} by using a lower BPA migration level (10 mg
9 BPA/kg resin) and assuming that the 1500 L wine vat is just lined with 1.4 kg
10 resin.
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19 According to the results obtained in the present study even this lower migration
20 level is an overestimation of the actual BPA concentrations in wine samples.
21 Based on the highest BPA level found in the present study (2.1 ng/ml), BPA
22 exposure due to wine consumption can be estimated to be lower than 26 ng/ kg
23 body weight/day, assuming a person with a body weight of 60 kg and a wine
24 consumption of 0.75 L per day. This value is about 400 times lower than the
25 Tolerable Daily Intake (TDI) for BPA of 0.01 mg / kg body weight/day proposed
26 by the SCF.
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35 Conclusion

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39 The highly selective analysis method presented allows the determination of
40 traces of BPA in wine samples without the interference of disturbing matrix
41 compounds. Its application to 59 wine samples revealed only very low BPA
42 concentrations. In 13 of the 59 wine samples the BPA concentration was lower
43 than the LOQ (0.2 ng/ml). The mean and median for all wine samples with BPA
44 concentrations above the LOQ were 0.58 and 0.40 ng/ml, respectively. These
45 values are far lower than the estimated BPA levels derived from migration
46 experiments using wine simulants.
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55 Acknowledgement

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3 We wish to thank Heidi Schwartz for carrying out the confirmation studies with
4 the HPLC system equipped with the electrochemical detector We are also
5 grateful to Georg Weingart for supplying wine samples from local vintagers
6 together with data on their storage conditions. The present study would have
7 been impossible without the support of Japan EnviroChemicals, Tokyo, Japan,
8 who provided BPA antibodies.
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Figures:

Fig. 1: Chromatograms of a BPA standard solution (A) and two wine samples (B,C) obtained after sample preparation by affinity chromatography: (A) BPA standard solution (10 ng/ml in ACN-water, 10:90, v/v); (B) white wine (sample 33, Table 3); C) red wine (sample 55, Table 3). HPLC system 1, injection volume 100 μ l.

Fig. 2: Chromatograms obtained after sample preparation by solid phase extraction: (A) white wine (sample 33, Table 2), (B) red wine (sample 55, Table 2).

Injection volume 100 μ l, HPLC system 1

Fig. 3: Migration of BPA from a plastic stopper. Chromatogram obtained by injecting 100 μ l aliquots of the ethanolic incubation solution into HPLC system 1.

Fig. 4: Migration of BPA from a plastic stopper. Chromatograms obtained by injecting into HPLC system 2 100 μ l aliquots of (A) a BPA standard solution (50 ng/ml in mobile phase) and (B) the ethanolic incubation solution.

Fig. 5: Current-voltage curves of the BPA standard solution (triangle) and the ethanolic incubation solution (square).

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Table 1: Analytical data

Wine	Sample volume (ml)	Standard addition correlation coefficient r	Mean recovery \pm SD (%) (n=3)	LOD (S/N=3)	LOQ (S/N=6)
White	25.0	0.9919	74 \pm 11	0.1 ng/ml	0.2 ng/ml
Red	25.0	0.9995	81 \pm 8	0.1 ng/ml	0.2 ng/ml

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Table 2a: BPA concentrations in wine samples taken directly from the vat. n.d.: below the LOQ (0.2 ng/ml)

Wine sample	Wine	Storage time (months)			BPA concentration (ng/ml)
		Steel vat	Wood vat	Plastic vat	
1	White	10	-	-	0.2
2	White	10	-	-	0.2
3	White	6	2	3	0.4
4	White	8	2	-	0.5
5	White	9.5	-	-	0.2
6	White	10.5	-	-	2.1
7	White	-	5	5	0.2
8	White	9.5	-	-	n.d.
9	Red	-	10	-	n.d.
10	Red	-	8	0.25	0.2

Table 2b: BPA concentrations in wine samples already filled into glass bottles. n.k.: storage time not known; n.d.: below the LOQ (0.2 ng/ml).

Wine sample	Wine	Volume (L)	Alcohol (%)	Top of the glass bottle	Storage time (months)				BPA concentration (ng/ml)
					Steel vat	Wood vat	Plastic vat	Glass bottle	
11	White	0.75	12.5	Plastic	5	-	-	11	n.d.
12	White	0.75	12.5	Cork	5	-	-	11	n.d.
13	White	0.75	12.5	Cork	8	-	-	5	n.d.
14	White	0.75	12.5	Plastic	8	-	-	5	n.d.
15	White	0.75	13.0	Cork	7	-	-	8	1.0
16	White	1.00	11.5	Metal	8	-	-	5	0.2
17	White	0.75	12.5	Cork	5	-	-	8	1.2
18	White	0.75	13.5	Cork	6	-	-	6	0.5
19	White	0.75	13.5	Plastic	6	-	-	6	0.4
20	White	0.75	12.5	Plastic	5	-	-	10	n.d.
21	White	1.00	12.0	Metal	3	2	-	9	0.6
22	White	1.00	11.5	Cork	-	3.5	3.5	7	0.6
23	White	1.00	11.5	Cork	n.k.	-	-	n.k.	0.5
24	White	0.75	12.0	Cork	n.k.	-	-	n.k.	1.5
25	White	0.75	13.5	Plastic	n.k.	-	-	n.k.	0.3
26	White	0.5	12.5	Plastic	n.k.	-	-	n.k.	0.2
27	White	1.00	12.5	Cork	-	-	n.k.	n.k.	0.3
28	Red	0.75	12.5	Cork	-	11	-	2	n.d.
29	Red	0.75	13.0	Cork	-	n.k.	0.25	n.k.	0.7
30	Red	1.00	11.5	Metal	9	-	-	6	0.8
31	Red	0.75	13.0	Plastic	-	n.k.	-	n.k.	0.4

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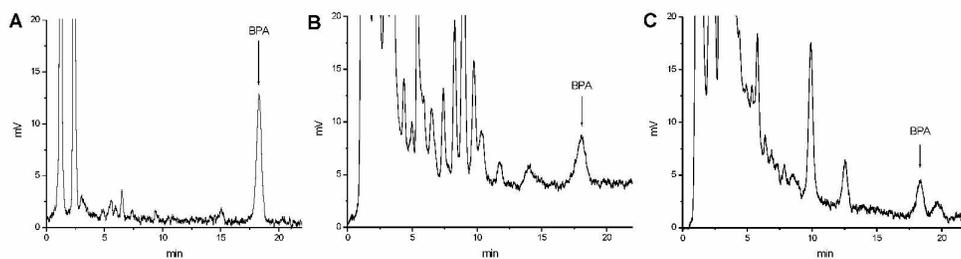
Table 3: BPA concentrations in wine samples bought in local markets. n.d.: below the LOQ (0.2 ng/ml).

Wine sample	Wine	Container	Volume (L)	Alcohol (%)	Top	BPA concentration (ng/ml)
32	White	Glass	1.00	12.5	Plastic	1.6
33	White	Glass	0.75	12.0	Plastic	0.2
34	White	Glass	0.25	11.5	Metal	0.3
35	White	Glass	0.25	11.5	Metal	0.3
36	White	Glass	0.38	12.5	Metal	0.3
37	White	Glass	0.75	11.5	Plastic	0.4
38	White	Glass	0.75	12.5	Metal	n.d.
39	White	Glass	0.75	12.0	Cork	0.3
40	White	Glass	0.75	11.5	Plastic	0.8
41	White	Glass	1.00	12.5	Cork	1.2
42	White	Tetra Brik	1.00	10.0	-	0.2
43	White	Tetra Brik	1.50	11.0	-	0.2
44	White	Tetra Brik	1.00	12.0	-	0.8
45	White	Tetra Brik	1.00	10.0	-	0.6
46	White	Tetra Brik	1.50	11.5	-	0.4
47	White	Tetra Brik	1.00	11.5	-	0.4
48	White	Tetra Brik	1.00	11.0	-	1.0
49	White	Tetra Brik	1.00	11.5	-	0.6
50	White	Tetra Brik	1.00	11.0	-	0.3
51	White	Tetra Brik	1.00	11.5	-	n.d.
52	White	Tetra Brik	1.00	11.5	-	0.4
53	Red	Glass	0.75	12.5	Cork	n.d.
54	Red	Glass	0.75	12.0	Plastic	0.5
55	Red	Glass	1.00	12.0	Metal	0.6
56	Red	Glass	0.75	13.0	Cork	n.d.
57	Red	Glass	0.75	13.5	Cork	1.2
58	Red	Glass	0.75	12.5	Cork	0.7
59	Red	Glass	0.75	12.0	Metal	n.d.

Table 4: Migration of BPA from plastic stoppers. n.d.: below the LOD (0.5 ng/ml).

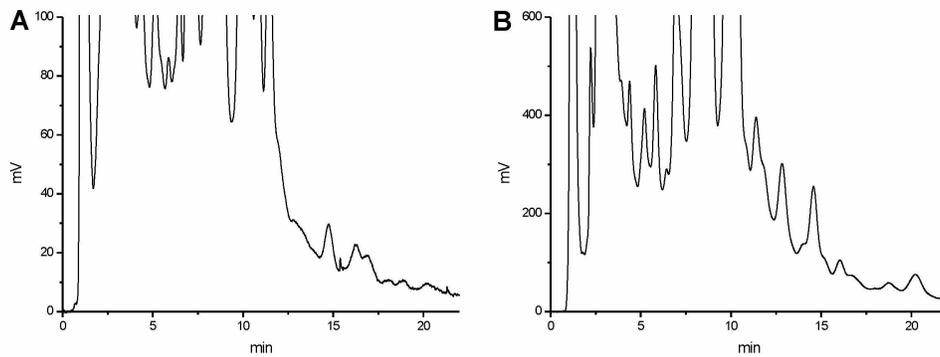
Stopper	Incubation period (weeks)	Mass of BPA migrated from the stopper (ng)
1	6	32
2	5	n.d.
3	11	n.d.
4	11	31

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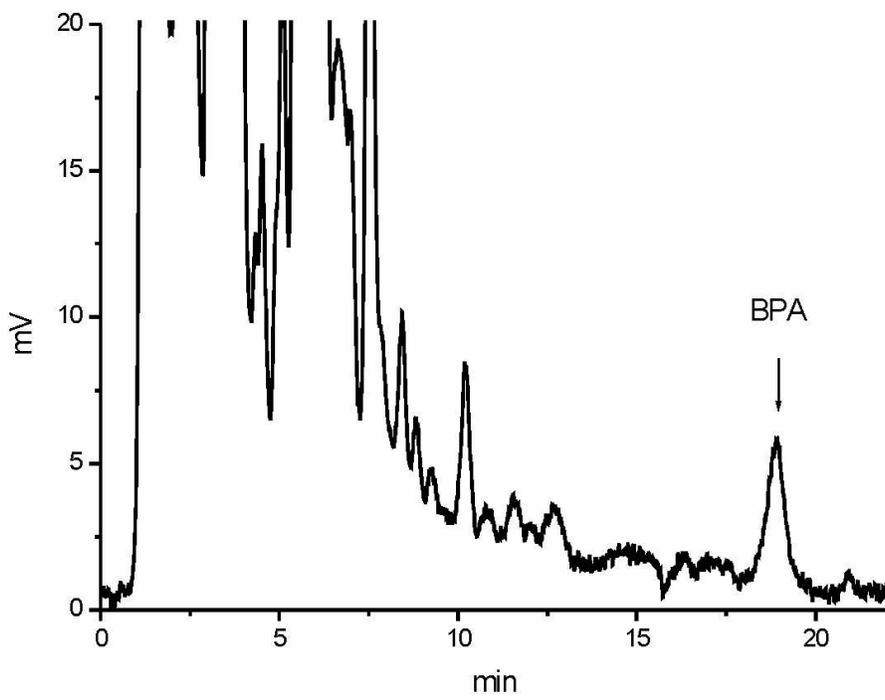


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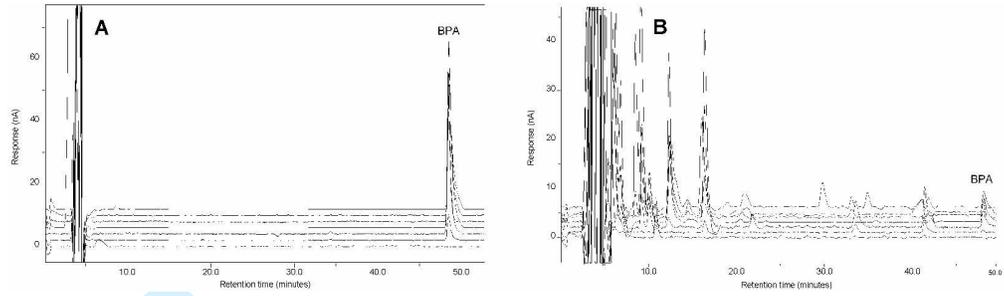
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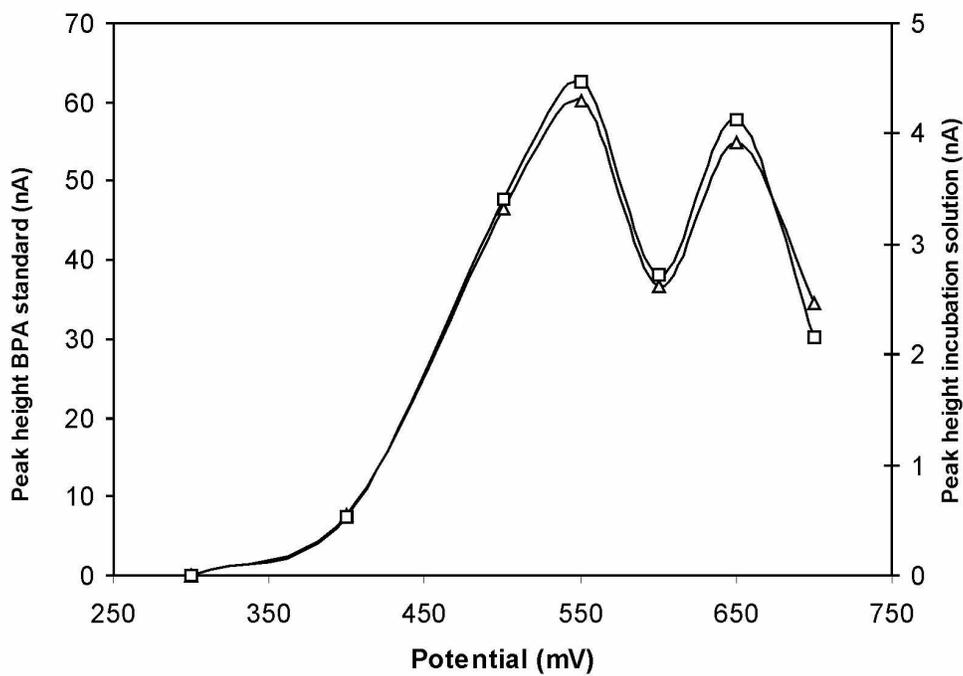
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