A sensitive ELISA for detection of the endocrine disruptor bisphenol A in water and milk

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Introduction

Bisphenol A (BPA) is used in large quantities for the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics have many applications including use in food and drink packaging, e.g. water- and baby bottles. Epoxy resins are used to coat metal products such as food cans, drink containers and water supply pipes. BPA can leach into food from the protective epoxy resin of canned food and from products such as food containers, water- and baby bottles.

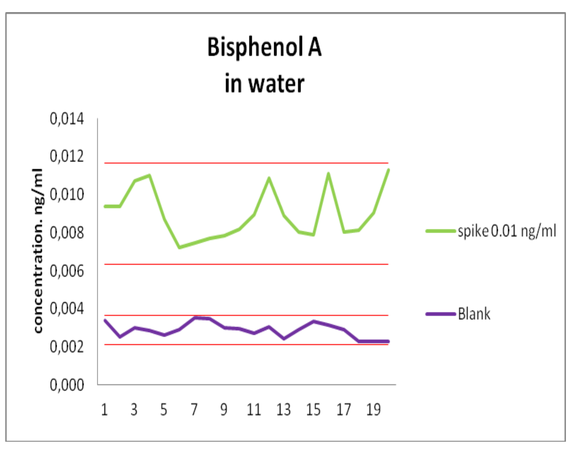
BPA is an endocrine disruptor, mimicking estrogens and thyroid hormones. Many studies have found that laboratory animals exposed to low levels of BPA show elevated rates of diabetes, mammary and prostate cancers, decreased sperm count, reproductive problems, early puberty, obesity, and neurological problems. In a draft scientific opinion report [1], the EFSA recommends the decrease of the tolerable daily intake (TDI) from 50 ppb bw/day to 5 ppb bw/day. At present there are no restrictions on the amount of BPA that can be present in a final plastic product, but the tendency of BPA to migrate from food contact materials has been acknowledged in the EU food law. EU legislation (2002/72/EC) [2] sets a Specific Migration Limit (SML) of 600 ppb BPA in food. The manufacture of BPA-containing baby bottles is prohibited since 2011 (2011/8/EU) [3]. The French National Assembly and Senate voted in 2012 to ban BPA from all food contact products by 2015.

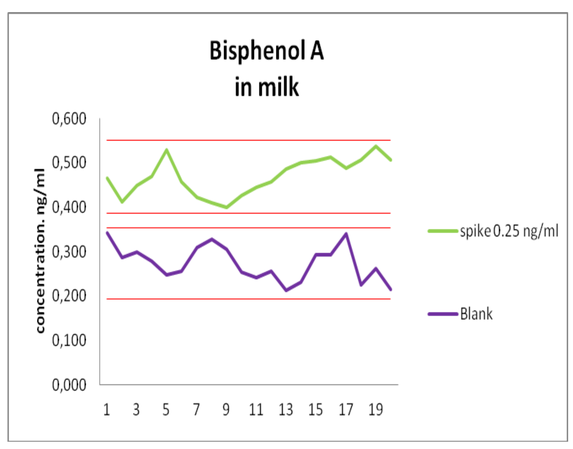
Using polyclonal rabbit antibodies, a competitive ELISA was developed for the detection of BPA in water and milk. Validation of this BPA ELISA was performed according to the European decision 2002/657/EC [4]. Objective of this validation study was to determine the detection capability (CCβ), limit of detection (LOD), cross-reactivity (specificity), precision (inter- and intra-assay variation), recovery, and stability of the ELISA.

Results

Detection Capability (CCβ)

Twenty blank samples of either water or bovine milk and 20 of such samples spiked with BPA were analysed in duplicate. The obtained CCβ values were 0.01 ppb for water and 0.25 ppb for milk.





Limit of detection (LOD)

Twenty blank samples of either water or milk were analysed in duplicate. The LODs were calculated as the mean value of these samples plus 3SD. The obtained LOD values were 0.005 ppb for water and 0.42 ppb for milk.

Cross-reactivity (antibody specificity)

The specificity of the polyclonal antibody was assessed by determining the extent to which the antibody cross-reacted with other compounds in buffer.

% Cross-reactivity = [ID50 of BPA/ID50 of competing compound] x100%.

The following cross-reactivities were obtained: 100% with BPA, 84.9% with 4,4-bis-(4-hydroxyphenyl) valeric acid, 6.0% with bis-(4-hydroxyphenyl) sulfone, 3.8% with 4,4'-cyclohexylidenebisphenol, 1.5% with 4-cumylphenol and <0.1% with bis-(4-hydroxyphenyl) methane, BPA-diglycidyl ether, coumestrol, dienestrol, diethylstilbestrol, estriol, estrone, hexestrol, zearalenone, β-estradiol 3-benzoate, β-estradiol 17-acetate, 17α-ethynylestradiol or nonylphenol.

Recovery

Six blank samples of either water or bovine milk were spiked with BPA and analysed in duplicate.

% Recovery = [spiked BPA conc./[mean found BPA conc.] x100%.

QC Criteria: 70-120%.

Sample	Sample preparation	Spike (ng/ml)	Abs. (450 nm)	Conc. (ng/ml)	Recovery (%)
Water	Extraction	0.025	0.874	0.021	84.0
Water	Extraction	0.050	0.661	0.040	80.0
Water	Extraction	0.075	0.524	0.067	89.3
Milk	Extraction	0.625	1.161	0.636	101.8
Milk	Extraction	1.25	0.876	1.345	107.6
Milk	Extraction	2.50	0.662	2.647	105.9

Precision (inter- and intra-assay variation)

For calculation of the CV_{inter}, standard solution of 1.0 ng/ml was tested 36 times in duplicate on 22 different days.

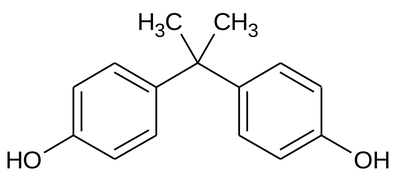
For calculation of the CV_{intra}, 6 blank samples of either water or milk, spiked with BPA, were analyzed in duplicate.

Sample	Conc. (ng/ml)	Abs. (450 nm)	σ (ng/ml)	CV_{inter} (%)
Standard	1.0	0.990	3.6	7.9

Sample	Spike (ng/ml)	Abs. (450 nm)	Conc. (ng/ml)	CV_{intra} (%)
Water	0.050	0.661	0.040	4.5
Water	0.075	0.524	0.067	4.4
Milk	1.25	0.876	1.345	4.0
Milk	2.50	0.662	2.647	3.9

Stability

In an accelerated stability study the ELISA was found to be stable for at least 12 months when stored at +4°C.



Chemical structure of BPA

Conclusions

- A sensitive ELISA was developed for the detection of BPA in water and milk at low ppb level.
- Validation of this BPA ELISA kit was performed according to the European Decision 2002/657/EC, determining the detection capability (CCβ), the limit of detection (LOD), cross-reactivity (specificity), precision (inter- and intra-assay variation), recovery, and stability.

References

1. EFSA DRAFT opinion on BPA exposure, 2014
2. Commission Directive 2002/72/EC. Official J. European Commun. L220 (2002) 18-58.
3. Commission Directive 2011/8/EU. Official J. European Union , L26 (2011) 11-14.
4. Commission Decision 2002/657/EC. Official J. European Commun. L221 (2002) 8-36.

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