APPLICATION NOTE

Mass Spectrometry

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Quantitation of BADGE: An Epoxyphenol-based Food Can Coating in Canned Tuna Extracts Using UHPLC-TOF

Introduction

Metal cans are often coated with a resin barrier to prevent contact between food and the can. Components from these coatings can migrate into the food affecting its safety and quality. Polyepoxyphenol coatings on the inside of cans based on bisphenol A epoxy resins can release the epoxy monomer bisphenol A diglycidyl ether (BADGE) into food (1,2). Bisphenol A and its derivatives are considered as endocrine disruptors (3). Both Europe and the U.S. have set regulations on the limit of BADGE migration into food at 1 mg/Kg. Using the quantitative capability of the AxION® 2 Time-of-Flight (TOF) mass spectrometer, we were able to set up a calibration curve and quantitate BADGE in a tuna extract. In addition, the high mass accuracy capability of the TOF along with the proprietary AxION EC ID software, allowed us to identify an unknown impurity cyclo-di-BADGE without having an authentic standard of this compound.

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Experimental

Sample preparation:

10 g of tuna was transferred into a 50 mL tube and spiked with BADGE standard (200 ng). To this, 10 mL of acetonitrile was added and shaken. Salts (1 g sodium chloride, 4 g magnesium sulfate, 1 g trisodium citrate, 0.5 g disodium hydrogen citrate) were added to the sample, which was shaken and centrifuged (3700 rpm) for 5 min. The supernatant (1 mL) was transferred to a dispersive SPE micro-centrifuge tube containing primary and secondary amine (PSA, 25 mg) and magnesium sulfate (150 mg) and C18 (25 mg). Sample was vortexed and centrifuged at 3000 rpm for 5 min. The supernatant was carefully removed, pH adjusted with 5 µL of 5% formic acid and used for analysis.

LC conditions:

Pump: PerkinElmer® Flexar™ FX-15 pump
Flow: 0.4 mL/min
Mobile phase A: Water containing 0.1% formic acid
Mobile phase B: Acetonitrile containing 0.1% formic acid
Gradient conditions: 70% A/30% B to 10%A/90%B in 5 mins in a linear gradient
Injection volume: 5 µL in partial fill mode.
Column used: PerkinElmer Brownlee™ SPP C-18, 2x50 mm, 2.7 µm, 25 °C

MS conditions:

Mass spectrometer: PerkinElmer AxION 2 TOF MS
Ionization source: PerkinElmer Ultraspray™ 2 (Dual ESI source)
Ionization mode: Positive
m/z range: 90-700
Capillary exit voltage: 100 V
Internal calibration was performed using *m/z* 118.08625 and 622.02896 as lock mass ions.

Results

The mass spectrum showed BADGE was predominantly observed as the [M+NH4+] ion (Figure 1). We were easily able to detect as low as 2 ppb concentration of BADGE (S/N = 52) standard. Excellent linearity (r^2 > 0.995) was observed for the calibration curve generated between 2 to 500 ng/mL (Figure 2) of BADGE standard. The intra assay %RSD for triplicate injections at the 2 ppb concentration was <10%. Tuna extracts spiked with 20 ng/mL of BADGE standard were easily detected by UHPLC-TOF (Figure 3). A 94% recovery of BADGE was observed in the spiked tuna extracts suggesting little or no ion suppression of the analyte in the extracts.

We tried to identify two unknown peaks with same exact masses eluting between 2.5 to 3 mins (Figure 4) using the exact mass capability of the AxION 2 TOF. The accurate mass and isotope profile was entered into the AxION EC ID calculator of the software. The software uses this

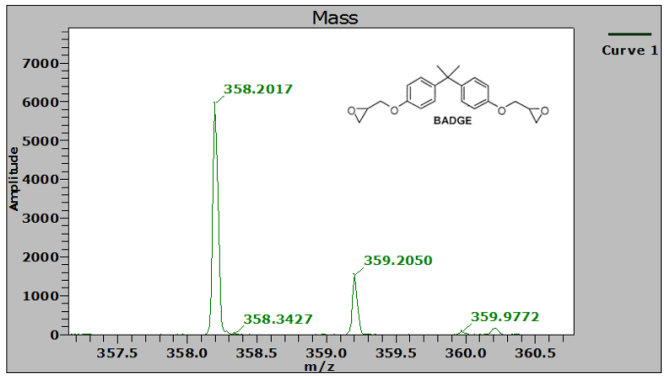

Figure 1. Mass spectrum shows [M+NH4]+ as the predominant peak for BADGE. Expected accurate mass of BADGE is 358.2013 (mass error < 2 ppm).

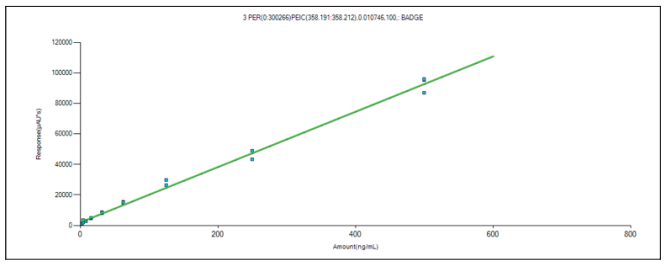

Figure 2. Shows calibration curve for BADGE analysis (r2 0.995).

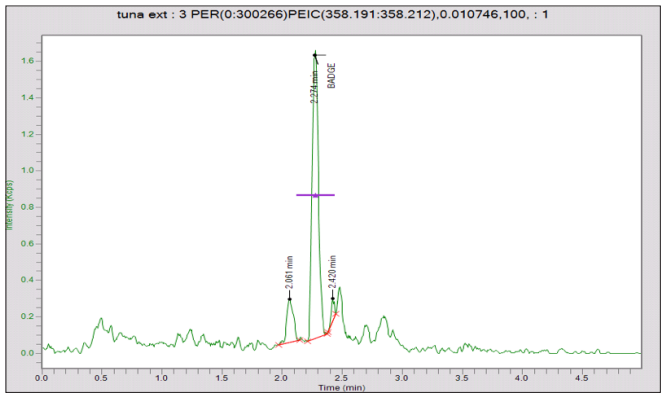

Figure 3. Analysis of BADGE by UHPLC-TOF in tuna extracts spiked at 0.2 mg/Kg .

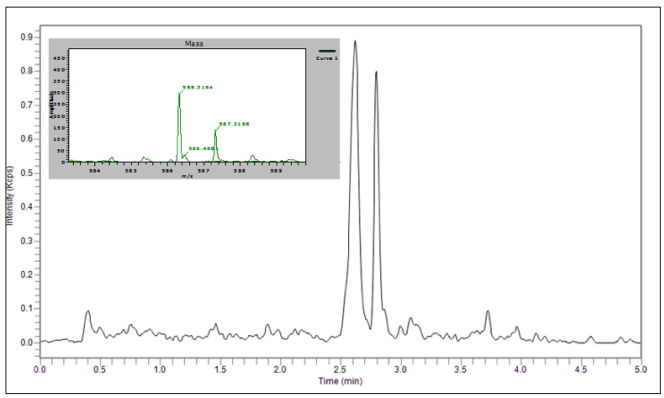

Figure 4. Unknown peaks in tuna extracts with the same *m/z* 586.3164 eluting at 2.6 and 2.8 min. Inset shows mass spectrum.

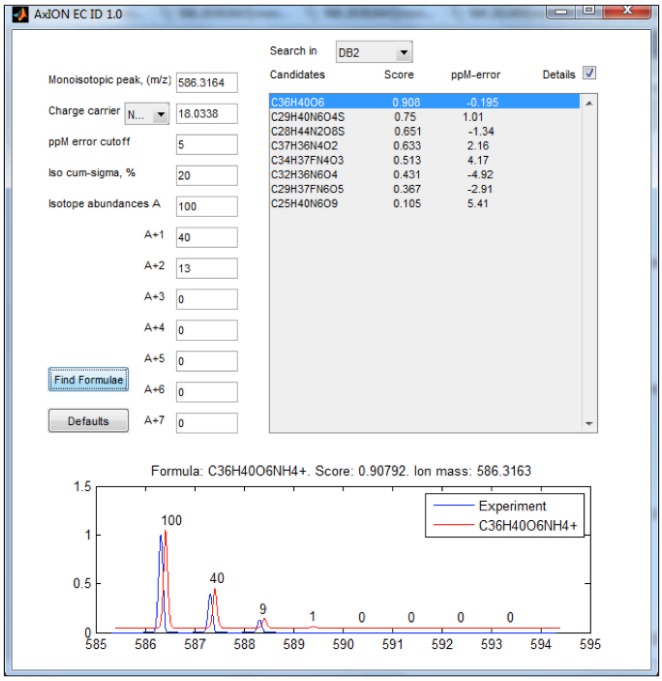

Figure 5. AxION EC ID software gave the elemental composition C36H40O6 as the top choice for the unknown mass 586.3164.

information and searches against the PubChem database and identifies potential molecular formulae matches. The first potential match with the highest score was identified with the elemental composition $C_{36}H_{40}O_6$ within a mass error of < 1 ppm (Figure 5). The software also provides a list of possible structures for the given elemental composition and one of the listed structures that related to bisphenol family of compounds was the BADGE.BPA linear structure (Figure 6a). However, an isomeric structure cyclo-di-BADGE compound described in the literature could also be possible (Figure 6b). Based on the fragmentation pattern and the retention time matching with an authentic standard, the presence of linear versus cyclo structure could be further confirmed.

Conclusion

Using the high sensitivity AxION 2 TOF, we were able to detect 0.2 mg/Kg of BADGE in tuna extract well below the regulation limits set at 1 mg/Kg. Using the high mass accuracy capability of AxION 2 TOF along with the AxION EC ID software, we were able to detect unknown peaks and match them to the isomers BADGE.BPA linear structure/cyclo-di-BADGE structures without the use of authentic standards.

References

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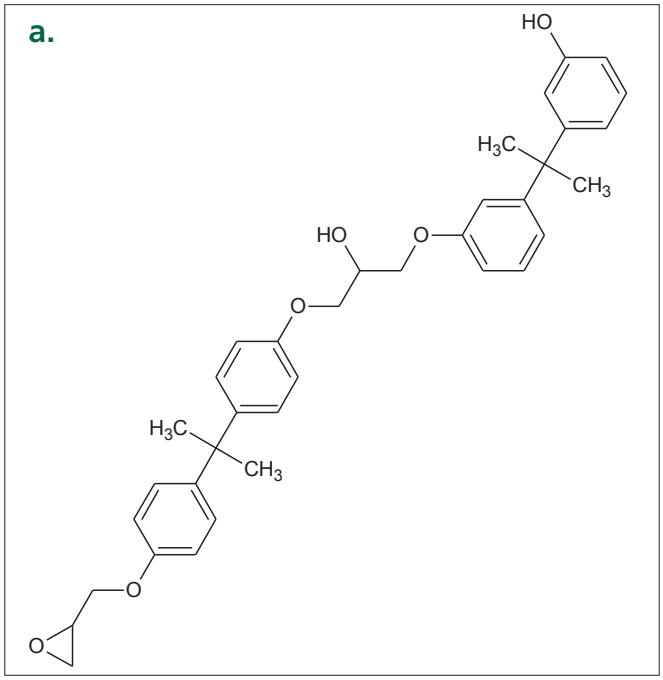
3. Schug T, Janesick A, Blumberg B, Heindel J Steroid Biochem Mol Biol. 127 (2011) 204.



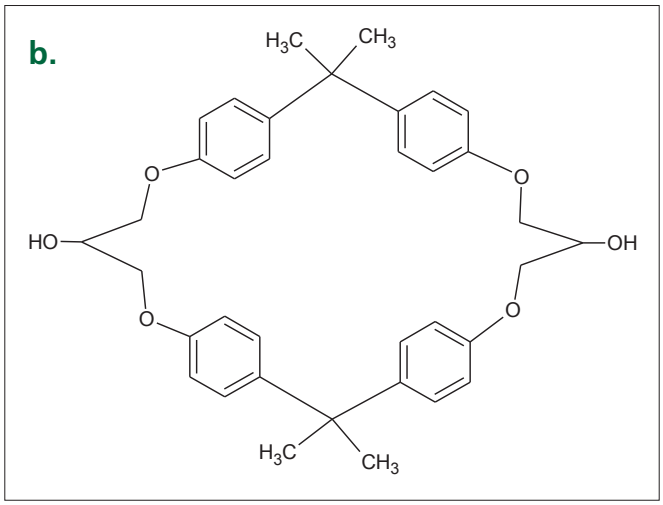


Figure 6 a and b. Structures that fit the elemental composition C36H40O6.
a. Linear BADGE.BPA
b. Cyclo-di-BADGE

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