

Analysis of Ethoxyquin and its Metabolites in Salmon Using QuEChERS

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Ethoxyquin (EQ) is a quinoline-based antioxidant, but it is still listed as a fungicide with pre- and post-harvest applications, mainly on pears, to reduce scald. It is also used in paprika powder and curcuma to reduce color loss due to oxidation of carotenoids. There are also numerous feed-related applications with indirect food relevance such as the use in fish feed to prevent lipid-oxidation. EQ is an approved feed additive in this field and is listed in the EU register on feed additives (Reg.1831/2003/EC) as E324. It has a permissible level of 150mg/kg in fish feed (Reg.2316/1998/EC). Fish is not currently regulated by Reg.396/2005/EC, so no maximum residue levels exist at this time. EQ degrades to a multitude of possible metabolites but only a few of them are commercially available as standards: Ethoxyquin-dimer (EQDM), 2,2,4-Trimethylquinolinon (QI) and Dihydroethoxyquin (DHEQ). EQ metabolites are valuable EQ-indicators especially in those cases, where routine multiresidue procedures lead to a complete or almost complete decomposition of the oxidation-prone parent.

Our studies mainly focused on salmon, and extractions were done by the QuEChERS method using 5g homogenate to reduce the influence of fat on the recoveries. As in a previous study on pears (see analytical observation on Ethoxyquin, EURL-SRM website) the impact of ascorbic acid (AA) was checked by adding it a) prior to cryogenic milling, b) prior to extraction, c) to the final extract and d) prior to cryogenic milling and additionally prior to extraction. The impact of AA on EQ extraction yields from farmed salmon was minimal. In contrast, AA showed a protective effect on EQ and especially on its QI when these were spiked on wild salmon. We assume that this is due to the high levels of other antioxidants in farmed fish such as carotenoids of plant origin and other antioxidants that are also frequently added to fish-feed. In recovery experiments on wild salmon, the metabolites EQDM and DHEQ were more stable and not notably affected by the addition of AA. Recoveries for QI could be improved from 11% (AA added to final extract) to 96% (AA addition to frozen salmon prior to QuEChERS extraction). Therefore the addition of AA prior to QuEChERS extraction is recommended.

Using AA in the extraction step validation of EQ, EQDM, DHEQ and QI in wild salmon showed satisfactory recoveries and variation coefficients. We recommend using freshly prepared matrix-matched calibrations because QI and DHEQ show strong matrix enhancement effects. Several salmon samples and shrimp samples from the market were analyzed. All farmed salmon samples contained both EQ and EQDM, but not QI and DHEQ. The concentrations of EQDM were significantly higher than those of EQ. None of the wild salmon and shrimps samples contained EQ or its metabolites.