XVIIIth International Symposium on Luminescence Spectrometry, Brest, France, June 2018.

Ultra-high performance liquid chromatography with fluorescence detection to determine benzimidazole compounds in farmed fish samples

[1] Carmen TEJADA-CASADO*, [1] Francisco J. LARA, [1] Ana M. GARCÍA-CAMPAÑA, [1] Monsalud DEL OLMO-IRUELA.

[1]*Dept. of Analytical Chemistry, University of Granada, Av. Fuente Nueva s/n, 18071, Spain.*

Introduction

Benzimidazoles (BZs) are veterinary drugs widely used as anti-parasitic agents in domestic animals. More recently, some BZs have also found applications in aquaculture to treat tape-worm infections. However, their widespread use could lead to BZ residues in animal-producing food, which may cause some negative effects on the consumer health, such as congenic malformations and teratogenicity among others. In this sense, the development of analytical methods to determine the presence of these residues is mandatory in order to fulfill European legislation, although for fish, maximum residue levels are not regulated yet.

Materials and Methods

UHPLC (Jasco-X-LC) separation was performed in a Zorbax Eclipse Plus RRHD C_{18} column (50×2.1 mm, 1.8 µm), involving a total analysis time lower than 12 min. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.4 mL/min. The column temperature was 40 °C and the injection volume 20 µL. Fluorescence detection was performed at maximum exc/em wavelengths: 316/400 nm for 5-OH-Thiabendazole (5-OH-TBZ); 290/325 nm for Albendazole 2-aminosulfone (ABZ-NH_2-SO_2); 280/320 nm for (Carbedazim)CBZ and (Benomyl) BEN; 290/325 nm for Albendazole sulfoxide (ABZ-SO), Thiabendazole (TBZ), Fuberidazole (FUB) and Albendazol sulfone (ABZ-SO_2); 290/340 nm for Oxibendazole (OXI) and 290/340 nm for Albendazole (ABZ), Triclabendazole sulfone (TCB-SO_2), Triclabendazole sulfoxide (TCB-SO) and Triclabendazole (TCB). Detector gain was set at 100.

Results and Discussion

The best separation in terms of resolution was achieved when MeCN was employed as organic solvent (B) in the mobile phase. Afterwards, water was selected as eluent (A) over 50 mM ammonium acetate. Although better resolution of TCB metabolites was achieved with ammonium acetate, it was discarded due to backpressure problems. On the other hand, mobile phase flow rate was studied from 0.4 to 0.6 mL min^{-1}, selecting 0.4 mL min^{-1} as optimum because higher flow rates involved a decrease of resolution between ABZ-SO and TBZ. The temperature of the column was evaluated from 30 to 50 ^{0} C, selecting 40 ^{0} C since all peaks were baseline resolved. Then, salting-out assisted liquid-liquid extraction (SALLE) was applied as sample treatment to different types of farmed fish (trout, sea bream and sea bass). To obtain satisfactory extraction efficiencies for the studied analytes, several parameters affecting the SALLE procedure were optimized including the amount of sample, type and volume of the extraction solvent, and the nature and amount of the salt used. Characterization of the method in terms of performance characteristics was carried out, obtaining satisfactory results in terms of linearity ($R^2 \geq 0.997$), repeatability (RSD≤6.1%), reproducibility (RSD≤10.8%) and recoveries (R≥79%; RSD≤7.8%). Detection limits ranged between 0.04 and 29.9 µg kg^{-1}.

Conclusion

This method has demonstrated to be suitable to determinate 13 BZs with high selectivity and sensitivity. Above all, it involves an easy, fast and inexpensive method for BZs monitoring in fish samples such as trout, sea bream and sea bass, allowing high extraction efficiency with low consumption of reagents, solvent and samples, and hence, it complies with Green Chemistry principles.

Acknowledgments

The authors gratefully acknowledge the financial support of the Andalusian Government (Junta de Andalucía) for supporting this work (Excellence Project Ref: P12-AGR-1647) and Spanish Ministry of Economy and Competitiveness (Project ref: AGL2015-70708-R).