HPLC DETERMINATION OF OCHRATOXIN A IN PIG TISSUES USING ENZYMATIC DIGESTION COUPLED TO MOLECULARLY IMPRINTED SOLID PHASE PURIFICATION

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Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite by various *Aspergillus* and *Penicillium* species with nephrotoxic, carcinogenic, immunotoxic and teratogenic potential effects (1). OTA has been found in several food commodities, including cereals, coffee, beer, wine and spices. OTA can also be present in food of farm animals as a result of carryover from animal contaminated feed (2); consequently, a permitted level of 1 µg/kg OTA in pig meat or pig-derived products was set in Italy, as in other countries. Conventional methods for the determination of OTA in animal tissues are performed by extraction with chloroform and followed by cleaning up with immunoaffinity columns or liquid-liquid partitioning (3). These procedures need a large amount of organic solvents which are environmentally harmful and hazardous to humans. The aim of the present study was to develop a new enzymatic digestion method coupled with molecularly imprinted solid phase purification (MISPE) for quantitative determination of OTA in pig tissues (muscle, liver, or kidney). Five grams of sample aliquot were homogenized with 5 ml of a phosphate buffer using an Ultra Turrax. A 2.5 g aliquot of the homogenate was transferred into a tube, incubated for 1 hour at 37°C with 10 ml solution of 1% pancreatin in a phosphate buffer, ultrasonicated at 75 Hz, purified with MISPE columns (pre-conditioned with 3 ml acetonitrile and 3 ml water). OTA elution was performed with methanol/acetic acid 98:2 (v/v). Final eluate was evaporated to dryness and re-dissolved into 1 ml of HPLC mobile phase and injected into HPLC-FLD. The method was validated according to EU criteria for the confirmatory methods for organic residues and contaminants (4). For all analyzed matrices mean recovery was > 89 %, intra and inter-day repeatability expressed as RSD < 5 % and LOD and LOQ were 0.0018 and 0.0054 µg/kg, respectively. The method can be applied as alternative routine procedure to detect OTA presence in pigs meat products.

(1) Malir et al. Ochratoxin A: 50 Years of Research. Toxins, 8:191, 2016. (2) Duarte et al. Ochratoxin A in feed and food-producing animals: an undesirable mycotoxin with health and performance effects. Vet. Microbiol. 154:1-13, 2011. (3) Monaci et al. Determination of ochratoxin A in pig tissues by liquid-liquid extraction and clean-up and high-performance liquid chromatography. Anal Bioanal Chem 378:1777-1782, 2004. (4) EC (2002) Commission Decision 657/2001 of 12 August 2002 Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. pp. 8-36.