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Determination of ochratoxin A in pig tissues using enzymatic digestion coupled with high-performance liquid chromatography with a fluorescence detector

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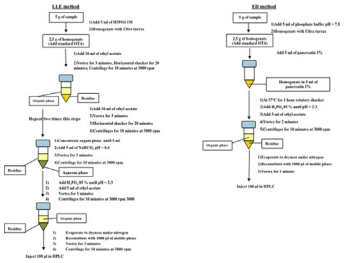
GRAPHICAL ABSTRACT



ABSTRACT

We present a new method for the rapid analysis of ochratoxin A (OTA) in pig tissues (muscle, liver and kidney) using enzymatic digestion (ED) coupled to high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). OTA was digested with a 1% pancreatin solution in a phosphate buffer and then cleaned with ethylacetate. After being evaporated to dryness and re-dissolved, the sample was determined using HPLC-FLD. The method was validated taking into account the currently permitted limit of 1 μg/kg OTA in pork meat and derived products in Italy. The recovery was higher than 90%. Intra- and inter-day repeatability expressed as RSD were less than 7%. The LOD and LOQ were 0.001 and 0.002 μg/kg, respectively. Our method is more efficient, easier, and cheaper than conventional clean-up procedures (liquid–liquid extraction).

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- The aim of the study was to develop and validate a quantitative HPLC-FLD method based on ED followed by a chromatographic analysis without any previous clean-up or concentration step for the detection of OTA in pig tissues.
- The ED method showed a 90%+ recovery, and intra- and inter-day RSD less than 7%.
- This method is simple, rapid, easy to use, and consumes low amounts of organic solvents.

ARTICLE INFO
Method name: Enzyme assisted digestion coupled to HPLC-FLD
Keywords: Enzyme assisted digestion, Ochratoxin A, Pig, Muscle, Kidney, Liver

Method details

Ochratoxin A (OTA) is a secondary toxic metabolite of various *Penicillium* and *Aspergillus* fungi, which is widely distributed in cereals [1]. OTA is nephrotoxic and immunotoxic. IARC classified OTA as a possible human carcinogen (Group 2B) [2]. Long-term exposure to OTA in humans has been implicated in Balkan endemic nephropathy (BEN) and is associated with urinary tract tumors because of the high OTA levels detected in food samples and in blood or urine from affected patients. As cereals are widely used in animal feed, animals are continuously exposed to OTA through the consumption of contaminated feed, which can lead to the accumulation of this mycotoxin in meat and meat products [3].

Some countries have set maximum levels of OTA in meat or animal products, such as Denmark (pig kidney 10 μg/kg, pig blood 25 μg/ml), Romania (pig kidney, liver, and meat 5 μg/kg), and Italy (pig derived products 1 μg/kg) [4]. As one of the main sources of meat for humans, it is essential to focus on the residues of OTA in pork. Given that mycotoxins have a particularly complex matrix, it is more difficult to determine them in meat than in cereal grains. The most common methods for the determination of OTA in animal tissues are performed by extraction with chloroform, followed by a clean-up with immunoaffinity columns or liquid–liquid partitioning [5–7]. However, conventional 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 and validate a new enzymatic digestion method coupled with HPLC-FLD for OTA quantitative determination in pig tissues.

HPLC-FLD analysis

The chromatographic system consisted of a Jasco 880 pump and a Jasco 821 fluorescence detector (Jasco, Tokyo, Japan). JascoBorwin software was used for data processing. The excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) were set at 380 and 420 nm, respectively. The reversed-phase column was a HAISIL HL, C_{18}, 5 μm, 150 mm × 4.6 mm (Higgins Analytical, USA). The column was kept at room temperature. The HPLC was operated with a mobile phase system consisting of a methanol-phosphate buffer solution pH 7.5 (0.03 M Na_2HPO_4, 0.007 M NaH_2PO_4) 50/50% v/v at flow rate of 1 ml/min.

OTA (from *Aspergillus ochraceus*) (M 403.8) reference standard was purchased from Sigma (Milan, Italy). The OTA standard was dissolved in a toluene-acetic acid mixture (99:1%, v/v) to give a stock solution of 200 μg/ml, which was stored at −20 °C until use. Working solutions were prepared by diluting the stock solution with the mobile phase consisting of a methanol-sodium phosphate buffer (pH 7.5) 50:50% v/v. HPLC-grade water, methanol, ethylacetate and acetonitrile were purchased from VWR (Milan, Italy). The pancreatin enzyme (from porcine pancreas) was purchased from Sigma (code P1750, Milan, Italy), and was stored at −20 °C until use.

Standard liquid–liquid extraction (LLE)

OTA was extracted according to Meucci et al. [8] with slight modifications. A 5 g liver, kidney or muscle sample aliquot was homogenized with 5 ml of phosphoric acid 1 M using an Ultra Turrax