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A rapid method for simultaneous determination of zearalenone, α-zearalenol, β-zearalenol, zearalanone, α-zearalanol and β-zearalanol in traditional Chinese medicines by ultra-high-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A rapid and reliable ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for simultaneous determination of zearalenone (ZEN), α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) in traditional Chinese medicines (TCMs) was developed. The development of the method and investigations of the matrix influence were described in particular. After evaluation of the matrix effects of different TCMs, i.e., rhizomes and roots, seeds, flowers, grasses and leaves, by the post-extraction spiked method, a reliable sample clean-up method based on home-made clean-up cartridges, a suitable internal standard and the matrix calibration were combined using to minimize the matrix effects to ensure the accuracy of the method. The established method was further validated by determining the linearity ($R^2 \geq 0.9990$), sensitivity (limit of quantitation 0.11–0.99 ng mL^{-1}), average recovery (86.6–113.5%) and precision (relative standard deviation ≤13.5%). It was shown to be a suitable method for simultaneous determination of ZEN, α-ZOL, β-ZOL, ZAN, α-ZAL and β-ZAL in different TCMs. Finally, the established method was successfully applied to the determination of the six mycotoxins in various TCMs and the results were presented to provide relevant insights to researchers in TCM analysis.

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1. Introduction

Zearalenone (ZEN) and its derivatives, α- and β-zearalenol (α- and β-ZOL) are a group of phenolic compounds produced by *Fusarium* moulds [1,2]. Acute and chronic injection of ZEN, α-ZOL and β-ZOL by animals can elicit a variety of toxic effects, i.e. enlargement of mammary glands and uterus, infertility, vaginal prolapse and atrophy of testicles and ovaries [1,3–5]. α-Zearalanol (α-ZAL) is a resorcyclic acid lactone and might arise from the metabolism of α-ZOL or ZEN [6,7]. It is currently used to improve feed conversion efficiency and promote growth rates in livestock production. However, long-term investigations with rats, dogs and monkeys indicate that α-ZAL is a weak oestrogen effecting predominantly changes in mammary glands and organs of the reproductive system [8]. The major metabolites of α-ZAL are zearalanone (ZAN) and β-zearalanol (β-ZAL), which also have endocrine-related biological activity though they are less biologically active than α-ZAL [6]. Because of the potential health effects of ZEN and its congeners in domestic animals (particularly swine), maximum residue limits (MRLs) for ZEN, ranging from 50 μg kg^{-1} to 1000 μg kg^{-1} in foods, have been established in many countries [9]. In the USA, the safe residue levels of α-ZAL in uncooked edible cattle tissues are set at 0.15 mg kg^{-1} in muscle, 0.3 mg kg^{-1} in liver, 0.45 mg kg^{-1} in kidney and 0.6 mg kg^{-1} in fat. In Australia, MRLs for α-ZAL are 0.005 mg kg^{-1} in cattle meat and 0.02 mg kg^{-1} in the edible offal of cattle [6].

In a recent report, ZEN and its derivatives were detected in maize collected from different farms placed in the north-center of Italy [10], indicating that this type of mycotoxins can contaminate natural samples. Although the presence of these mycotoxins in natural samples is potentially harmful, only several papers monitored of traditional Chinese medicines (TCMs) and were only directed towards ZEN [11], whereas other mycotoxins such as α-ZOL, β-ZOL, α-ZAL, β-ZAL have received no attention. As the safety of TCMs has attracted more and more attention because of the number of people using such products as alternative therapy or self-medication increasing, development and validation of a reliable method for simultaneous determination of ZEN, α-ZOL, β-ZOL, ZAN, α-ZAL

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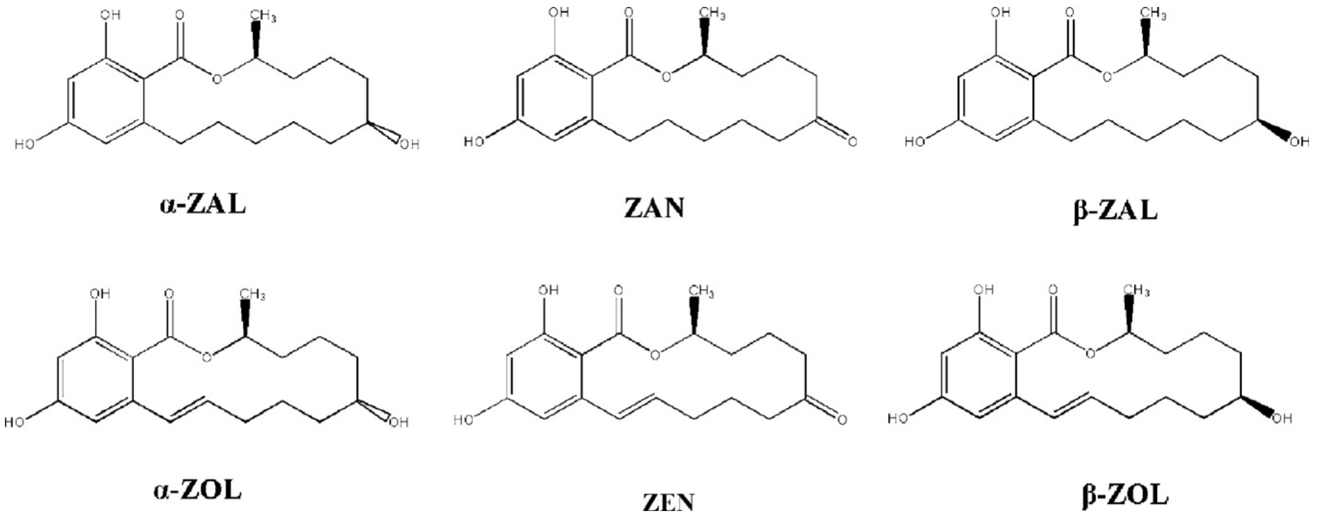

Fig. 1. Chemical structures of ZEN, α-ZOL, β-ZOL, ZAN, α-ZAL and β-ZAL.

and β-ZAL in TCMs are essential issues to ensure their safe uses.

The research on the methods with the employment of adequate extraction and clean-up procedures has been promoted by the increasing need for multi-mycotoxin analysis. Based on the physico-chemical properties of these mycotoxins, the samples are extracted with mixtures of methanol/water (sometimes added with NaCl) or acetonitrile/water (acetonitrile 75–85%, v/v) [10,12–14]. For further purification and analyte enrichment, the extracts are submitted to solid-phase extraction (SPE) with a variety of different sorbents, i.e., florisil, graphitized carbon black, carbograph-4 or immunoaffinity materials [15–17]. However, there are no uniform methods, to be generally employed for the given application to TCMs, due to the variety of the matrices, along with their complex composition.

Due to the widespread occurrence and trace amount of these mycotoxins, rapid, sensitive and reliable quantitative methods are required. Thin-layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA) methods are widely used screening tests [18–22]. However, low sensitivity, unsatisfied accuracy and poor separation efficiency limit the application of TLC. ELISA-based kits, which are generally expensive, may suffer from cross-reactivity phenomena giving rise to false positive results. Although the GC method has a good sensitivity and specificity, the major disadvantage is the need for derivatization, which is time-consuming, to enhance the volatility of analytes for GC separation [14,23–28]. The most frequently used technique for the mycotoxin separation is HPLC as it combines high resolution with increasing sophisticated automation [10,12,21]. The availability of ionization sources, i.e., electrospray (ESI) and atmospheric pressure chemical ionization (APCI), has drastically improved the possibilities of employing LC/MS in the mycotoxin analysis [29,30]. In addition, tandem mass spectrometry (MS/MS) results in enhanced performance, providing additional selectivity and generating information with a high degree of structural specificity [15,31–35]. However, the common perception that utilization of LC–MS/MS practically guarantees selectivity is challenged by several reported examples of lack of selectivity due to ion suppression (or enhancement) caused by the sample matrix. Possible strategies including the use of matrix matched standards or performing a standard addition protocol can amend these effects. Both of the two approaches are not suitable for the analysis in the present study as there are so many categories of TCMs. The most effective and accurate way to ensure the analytical results is the stable isotope dilution assays (SIDA). The big advantage is that the isotope internal standards (ISs) behave similar to the target analytes during the whole sample preparation and ionization process, but can be distinguished by their different molecular weight in the mass spectrometer. The big obstacle to wide use of SIDA is that the isotope IS of relative mycotoxin as commercial-product is very difficult to be obtained. Hitherto, there are no reports about the determination of ZEN, ZAN, α-ZOL, β-ZOL, α-ZAL and β-ZAL in various TCMs by LC–MS/MS using the isotope dilution method.

The aim of this study is to develop a rapid and reliable UHPLC–MS/MS method, using [$^{13}C_{18}$]-ZEN as the IS, for simultaneous determination of ZEN, ZAN, α-ZOL, β-ZOL, α-ZAL and β-ZAL in various TCMs, mainly considering the actual contaminant situations in Zhejiang province in China.

2. Experimental

2.1. Chemicals

The standards including ZEN, ZAN, α-ZOL, β-ZOL, α-ZAL and β-ZAL, as well as [$^{13}C_{18}$]-ZEN (IS), were purchased from Sigma–Aldrich (St. Louis, MO, USA). The chemical structures of the six mycotoxins are shown in Fig. 1. QC material (ZEN in corn, ~264 $\mu g\,kg^{-1}$) was purchased from Romer Labs (Washington, MO, USA).

Acetonitrile and methanol, both HPLC grade, were obtained from Merck (Darmstadt, Germany). Other chemicals and solvents were of HPLC or analytical grade. Deionized water was purified using a Milli-Q Gradient A 10 system (Millipore, Billerica, MA, USA).

Alumina base, florisil, silica gel, alumina acid, alumina neutral and kieselguhr were purchased from Sigma–Aldrich (St. Louis, MO, USA). High quality poly (9,9-diethylfluorene) syringe filters (0.22 μm pore size, 13 mm diameter) were purchased from Millipore (Billerica, MA, USA). Polypropylene 6 mL SPE empty tubes and cribriform plates were purchased from Shenzhen Biocomma Biotech CO, Ltd.

2.2. Apparatus

UHPLC was performed using a Waters Acquity Ultra-high-performance LC system (Waters, Milford, MA, USA). The six mycotoxins were completely separated on an Acquity UPLC BEH Shield RP18 column (150 mm × 2.1 mm, 1.7 μm) at 45 °C, with a mobile phase flow rate of 0.35 $mL\,min^{-1}$. The mobile phase con-

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