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Determination of dinitolmide and its metabolite 3-ANOT in chicken tissues via ASE-SPE-GC–MS/MS

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ABSTRACT

A novel confirmatory analysis method to simultaneously extract, clean-up and detect dinitolmide and its metabolite (3-amino-2-methyl-5-nitrobenzamide, 3-ANOT) in chicken tissues using accelerated solvent extraction (ASE) with a neutral alumina solid-phase extraction (SPE) column coupled with gas chromatography-tandem mass spectrometry (ASE-SPE-GC–MS/MS) was established. GC–MS/MS was performed using a VF-5ht capillary column with helium as the carrier gas, temperature programming, and an inlet temperature of 340 °C. The experimental method was carried out in the electron ionization mode; the full scan mode was used for qualitative analysis, and selective reaction monitoring, combined with an external standard method was used for quantitation. Under the optimized conditions, at the addition levels of the limits of quantitation (LOQs), 0.5 MRL (maximum residue limit), 1 MRL and 2 MRL, the average recoveries of dinitolmide and 3-ANOT in chicken tissues were 81.96–94.31%, and the relative standard deviations (RSDs) were 1.72–5.37%. The limits of detection (LODs) (S/N ≥ 3) of dinitolmide and 3-ANOT in chicken tissues were 0.8–2.5 μg/kg, and the LOQs (S/N ≥ 10) were 2.7–8.0 μg/kg. The method validation parameters met the Chinese Ministry of Agriculture, European Union and United States Food and Drug Administration veterinary drug residue detection requirements.

1. Introduction

Dinitolmide (zoalene; 3,5-dinitro-*o*-toluamide) was researched and developed by the Dow Corporation in France in 1960. Dinitolmide is a commonly used nitro amide antiprotozoal agent in poultry farming because of its low toxicity, stability, low cost and anticoccidial effects (He et al., 2012). However, long-term use and excessive concentrations have caused most of the veterinary drug intake by chickens to be excreted in the form of a prototype drug or metabolite that not only pollutes the environment but also produces veterinary residues in edible animal tissue, endangering human health (Beyene, 2016). Therefore, to improve the safe management of veterinary drugs, protect the environment, and ensure the safety of animal-based food and human health, the U.S. Food and Drug Administration (2014) and Chinese Ministry of Agriculture (2002) stipulate a maximum residue limit (MRL) of dinitolmide and its metabolite 3-amino-2-methyl-5-nitrobenzamide (3-ANOT) in poultry tissue. The MRLs of dinitolmide and 3-ANOT in animal tissues specified by the U.S. Food and Drug Administration and Chinese Ministry of Agriculture are consistent: chicken muscle, 3000 μg/kg; liver and kidney, 6000 μg/kg; skin and fat, 2000 μg/kg. Therefore, the establishment of a confirmatory analysis method for dinitolmide and 3-ANOT is of great value.

The primary methods of detection for dinitolmide and 3-ANOT in chicken tissue are high-performance liquid chromatography (HPLC) (Cody et al., 1990; Nagata and Saeki, 1988; Parks and Doerr, 1986), ultra-performance liquid chromatography (UPLC) (Wu et al., 2011a), high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) (Cronly et al., 2010; Wei et al., 2015; Zhao and Wu, 2011) and ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) (Muharem et al., 2015; Wu et al., 2011b). Most of these methods use manual extraction to assess the samples, and the steps are numerous and time consuming. However, the use of

Abbreviations: GC–MS/MS, gas chromatography–tandem mass spectrometry; ASE, accelerated solvent extraction; SPE, solid-phase extraction; LOD, limit of detection; LOQ, limit of quantitation; MRL, maximum residue limit; SRM, selective reaction monitor; FDA, Food and Drug Administration; RSD, relative standard deviation

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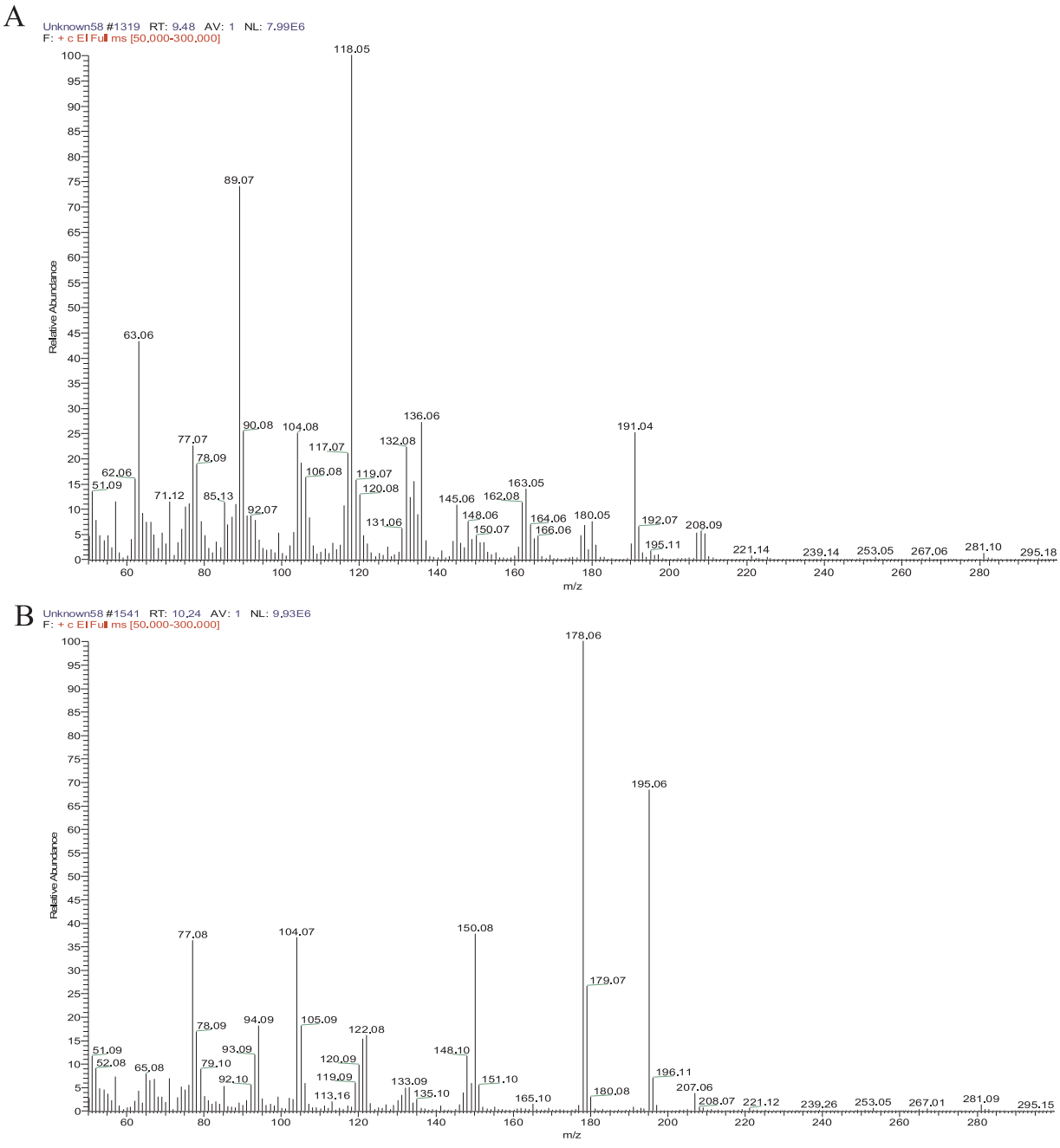

Fig. 1. Mass spectra of dinitolmide (A) and 3-ANOT (B) reference substances.

modern sample pretreatment equipment, including accelerated solvent extraction (ASE) and centrifugal concentrator combined with gas chromatography-tandem mass spectrometry (GC–MS/MS) for the simultaneous extraction and determination of dinitolmide and 3-ANOT residues in chicken tissue has not been reported. Compared with traditional extraction methods, ASE has the advantages of less solvent, fast extraction, high efficiency, high recovery, good reproducibility and a high degree of automation. Meanwhile, compared with LC–MS/MS, the ion source of GC–MS/MS is an electron ionization (EI) source. The obtained ion fragment information of the compounds is more abundant and has a mature spectral library to facilitate the search of compounds, which is convenient for the identification of compounds. Additionally, GC–MS/MS instruments are cheaper than LC–MS/MS instruments.

Thus, the purpose of this study was to establish and optimize an ASE-SPE-GC–MS/MS confirmatory analysis method to simultaneously extract, clean-up and detect dinitolmide and 3-ANOT from chicken tissues. Moreover, this study employs a capillary column with high temperature resistance and without a cumbersome derivatization process that can directly detect dinitolmide and 3-ANOT. This method is fast and allows accurate quantification, high recoveries and good sensitivity. The methodology parameters meet the European Union (2002/657/EC, 2002) and US Food and Drug Administration (U.S. Department of Health and Human Services et al., 2001) veterinary drug residue detection requirements. This study provides a new method for the detection of dinitolmide and 3-ANOT in animal-derived tissues, offering a scientific basis for the development of drug residue detection standards in animal-derived foods.