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Simultaneous determination of amantadine, rimantadine and memantine in chicken muscle using multi-walled carbon nanotubes as a reversed-dispersive solid phase extraction sorbent

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ABSTRACT

A modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method using multi-walled carbon nanotubes (MWCNTs) as a reversed-dispersive solid phase extraction (r-dSPE) material combined with ultra-high liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) was developed for the simultaneous determination of amantadine, rimantadine and memantine in chicken muscle. The satisfactory separation of isomers (rimantadine and memantine) was obtained on an Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) after optimization of mobile phase composition, column temperature and flow rate. The method involved an acetonitrile-based sample preparation and a dSPE clean-up procedure with MWCNTs material. Variations in the type and amount of MWCNTs, the pH value of the extract, the extraction time for MWCNTs, and the type of eluent were used to determine the optimal parameters for increasing the sample throughput and the sensitivity. The samples were quantified using amantadine-D_{15}, rimantadine-D_4 and memantine-D_6 as the internal standards. Under the optimized conditions, recoveries of 96.8–104.6% and the values of coefficient of variation (CV) of 3.8–6.4% were obtained for the three drugs in chicken muscle at three spiked levels (0.5, 1.0 and 1.5 μg/kg), and the decision limits (CCα) and detection capabilities (CCβ) were 0.15–0.20 μg/kg and 0.20–0.25 μg/kg, respectively. Positive results were obtained from local supermarket using this method, and the concentrations obtained from the newly developed method compared well to the previously reported method.

1. Introduction

Amantadine (AT) was the first drug approved for prophylaxis of influenza type A, and in 1976, it was approved for treatment and prophylaxis in adults and children older than one year [1]. Rimantadine (RT) was approved in 1993 for treatment and prophylaxis of influenza type A infection in adults [2]. However, the widespread use of AT and RT has resulted in the rapid emergence of drug-resistant variants [3–5]. Drug resistance will affect the effectiveness of human optional drugs against the influenza virus and may significantly enhance the risk of cross infection between animals and humans. For example, the avian influenza virus can affect both birds and mammals, including humans [6,7]. Because of the potential for cross infection, the application of the adamantane drugs is banned in animal husbandry to protect consumers in many countries, including the USA [8] and China. However, there is evidence that the adamantane drugs include memantine (MT), which is a tricyclic amine chemically and pharmacologically related to the antiviral prototype AT, have illegally been used for the prevention and treatment of influenza A virus infections in animal husbandry in China [9,10]. Therefore, there is a need for the development of a simple, rapid, specific, inexpensive, and sensitive method to detect the presence of the adamantane drugs in chicken.

A number of methods have been reported for the determination of AT, RT, and MT in biological samples, including gas chromatography (GC) [11], gas chromatography mass spectrometry (GC–MS) [12–14], liquid chromatography (LC) [15–17], capillary electrophoresis (CE) [18,19], and liquid chromatography–mass spectrometry (LC–MS and LC–MS/MS) methods [9,10,20–24]. However, it is necessary to make derivatives of the three drugs to enhance their volatility for GC or GC–MS analysis and to increase instrument sensitivity for LC and CE analysis. Compared to the other instrumental methods, the LC–MS/MS method allows for direct determination of the three drugs due to its greater selectivity and sensitivity. Due to RT and MT are isomers and daughter ions of the two drugs are nearly the same (Fig. 1), there is interference between

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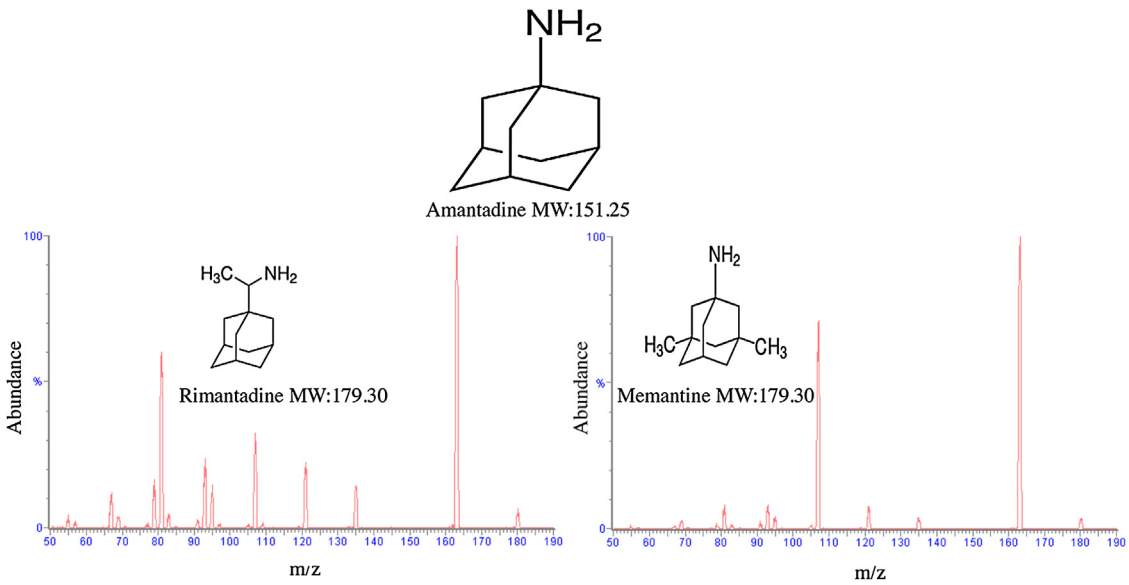

Fig. 1. The chemical structures of AT, RT, and MT and mass spectra of RT and MT.

them if they have not been effectively separated before entering a mass spectrometer. However, the phenomenon has been ignored for the established LC–MS/MS methods [10].

In recent years, a few LC–MS/MS methods have been developed for the determination of AT, RT, and MT in chicken tissues [9,10,23,24]. An LC–MS/MS method for determining seven antiviral drugs including AT and RT in poultry muscle has been developed by Berendsen et al. [23] with good sensitivity and accuracy, but the sample preparation involves multiple solid phase extraction (SPE) steps and is very time-consuming. Recently, Liu et al. [10] developed a rapid LC–MS/MS method for the determination of five antiviral drugs including AT, RT, and MT in chicken tissues, but the sample preparation still includes a conventional SPE procedure. These time-consuming sample preparation steps are required because of the difficulties encountered when analyzing the three drugs in complex samples. To improve the sample throughput for the analysis of antiviral drugs including AT and RT in chicken muscle, a novel quick, easy, cheap, effective, rugged, and safe (QuEChERS) method using acetonitrile (1% acetic acid, v/v) as extraction solution and C_{18} sorbent for clean-up has been developed for use with ultra-high performance liquid chromatography coupled with high resolution LTQ Orbitrap mass spectrometry [24]. The method has very good limits of detection (1.02 μg/kg and 0.67 μg/kg for AT and RT, respectively) and accuracy (82.5–105.8%). However, the use of multi-walled carbon nanotubes (MWCNTs) as a dispersive solid phase extraction (dSPE) material has not been reported for the determination of antiviral drugs including the three drugs in food.

Carbon nanotubes (CNTs) are materials made of carbon molecules and were discovered in 1991 by Iijima [25]. Due to their remarkable physical and chemical properties, CNTs have attracted more research interest than any other material and have been used in many fields [26]. In recent years, some groups have reported on the feasibility of using MWCNTs as part of a dSPE method for the determination of pesticides and veterinary drugs, and the results have shown high recoveries due to the efficient adsorption properties of MWCNTs [27–30]. As a result, it is feasible to develop high throughput methods based on MWCNTs as a dSPE material due to their fast and simple pre-treatment procedure.

In this paper, we describe a simple and sensitive LC–MS/MS method for the determination of AT, RT, and MT in chicken muscle using MWCNTs as a dSPE material. RT and MT have been effectively separated on an Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm). The type and amount of MWCNTs, the pH value of the extract, the extraction time with the MWCNTs, and the type of eluent have been optimized. The validation parameters tested include linearity, specificity, CCα, CCβ, recovery, precision and stability. The method is suitable for the routine determination of the three drugs in chicken samples through method validation studies.

2. Materials and methods

2.1. Materials and reagents

Methanol (LC grade) and acetonitrile (ACN, LC grade) were obtained from Fisher Scientific (Fairlawn, USA). Formic acid (LC grade) was obtained from Tedia Company Inc. (Fairfield, USA). Hydrochloric acid, sodium hydroxide, and acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). AT hydrochloride, RT hydrochloride, and MT hydrochloride were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Amantadine-D_{15} hydrochloride, rimantadine-D_4 hydrochloride, and memantine-D_6 hydrochloride were purchased from Toronto Research Chemicals (North York, ON, Canada). Five types of MWCNTs with similar lengths (10–30 μm or 10–20 μm) and different outer diameters (MWCNT01 < 8 nm, MWCNT02 = 10–20 nm, MWCNT03 = 20–30 nm, MWCNT04 = 30–50 nm, and MWCNT05 > 50 nm) were purchased from Nanjing XF NANO Materials Tech Co. Ltd. (Nanjing, China). The specific surface areas of the MWCNTs were 500, 200, 110, 60, and 40 m^2/g for MWCNT01, MWCNT02, MWCNT03, MWCNT04, and MWCNT05, respectively. The water was purified with a Milli-Q reverse osmosis system (Millipore, Milford, Massachusetts, USA).

2.2. Standard solutions

Individual stock solutions of 6 compounds (100 μg/mL) were prepared in methanol. Three spiking mixed standard solutions of AT, RT, and MT (20, 40, and 60 μg/L) were prepared by diluting the stock standard solutions with ACN. One mixed internal standard working solution of amantadine-D_{15}, rimantadine-D_4, and memantine-D_6 (100 μg/L) was prepared in ACN. Six individual standard working solutions (1000 μg/L for 6 compounds) for