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# Determination and pharmacokinetic studies of arecoline in dog plasma by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for the determination of arecoline concentration in dog plasma. Plasma sample was prepared by protein precipitation using *n*-hexane (containing 1% isoamyl alcohol) with β-pinene as an internal standard. Chromatographic separation was achieved on an Agilent C18 column (4.6 × 75 mm, 3.5 μm) using methanol: 5 mM ammonium acetate as the mobile phase with isocratic elution. Mass detection was carried out using positive electrospray ionization in multiple reaction monitoring mode. The calibration curve for arecoline was linear over a concentration range of 2–500 ng/mL. The intra-day and inter-day accuracy and precision were within the acceptable limits of ±10% at all concentrations. In summary, the LC–MS/MS method described herein was fully validated and successfully applied to the pharmacokinetic study of arecoline hydrobromide tablets in dogs after oral administration.



## 1. Introduction

Taeniasis, a zoonotic parasitic disease, will cause serious health risk to humans and animals. The taeniasis/cysticercosis disease complex has been recognized as a re-emerging disease in both developed and developing countries [1,2]. Since 1990, several joint collaborative projects in China have been initiated to address not only the cestode zoonoses but also many other parasitic and/or infectious diseases [3]. Human cases of taeniasis and cysticercosis have been reported in Asia and the Asia–Pacific regions [4,5]. According to statistics, currently, dogs infected with Taeniasis-causing tapeworms in China and the Testour Region in Tunisiamore account for more than 30% and 27%, respectively [6]. Despite the large numbers of studies on this disease, efficient drugs to treat taeniasis remain to be developed. Praziquantel [7] was the most commonly used drug to treat taeniasis, however, its popularity has been lessened due to its pungent odor, which makes it difficult for infected animals to swallow. Additionally, niclosamide, as an alternative drug, is severely toxic to some aquatic organisms [8,9].

Arecoline is a natural product, extracted from the plant species of *Areca catechu* L. Arecoline has antiparasitic, antifungaland antiviral effects, and is able to dissolve food stagnation and promote urination [10]. It has been widely used for treatment of tapeworm infestation, abdominal distension, diarrhea and edema [10].

Arecoline has high efficiency, low cost, and low toxicity, and is used in fruit form. However, the content of arecoline in extract from *A. catechu* L. is only 0.3% to 0.7% [11], which coupled with the limited sources of medicinal herbs and low efficiency of traditional extraction methods greatly limits its clinical application. On the other hand, arecoline as liquid is not convient to adminster, its' salt is appropriate for clinic use. No formulations of arecoline in salt forms are currently available for clinical use. In our previous study, we have successfully synthesized bulk drug—arecoline hydrobromide and prepared arecoline hydrobromide tablets as an oral preparation for the first time. Acute toxicity studies indicated that the toxicity of arecoline is four times higher than that of arecoline hydrobromide, therefore, we synthesized the hydrobromide formulation [12]. The proposed synthesis process can achieve a yield of more than 78% [13]. The benefits of this new technology include not only high yield, but also easy purification of the product, simple process operation, and easy scale-up for industrial

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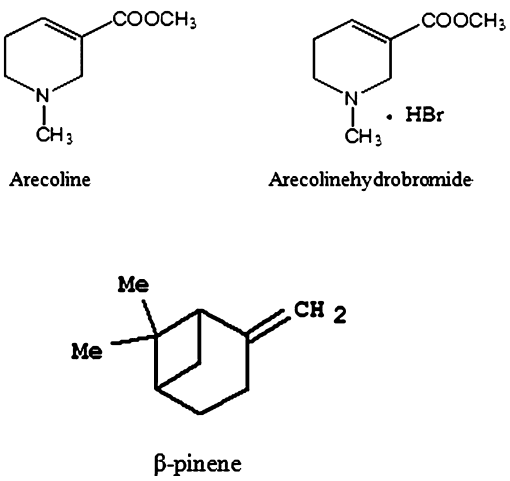


**Fig. 1.** Chemical structures of arecoline and arecoline hydrobromide.

production [13]. Both arecoline hydrobromide drug and tablet are nontoxic and safe for clinical use according to acute toxicity studies of oral bulk arecoline hydrobromide drug and tablets in rats and mice [14,15]. In addition, arecoline hydrobromide can enhance bowel movements and increase digestive gland secretion for more rapid elimination of parasites [16], and has significant anthelmintic effects on tapeworms in dogs [17].

In order to define the pharmacokinetic profile of arecoline hydrobromide tablets, a new method for the characterization of arecoline is needed. As a result, we propose to use high performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) to characterize the pharmacokinetics of arecoline in dog plasma. Previous attempts to characterize arecoline have mainly included acid–base titration, spectrophotometry, ion exchange chromatography [18], capillary electrophoresis (CE) [19], and high performance liquid chromatography [20]. Because arecoline contains no conjugated groups in the structure (Fig. 1), it has no appropriate chromophores for use in characterization techniques. Its maximum wavelength for ultraviolet absorption is 215 nm, which is near the end of the absorption range, and the sensitivity is thus low [21]. Therefore, we chose to develop a LC–MS/MS method for determining the concentration of arecoline in dog plasma. LC–MS/MS is accurate and sensitive, and requires only a simple protein precipitation pretreatment for arecoline with β-pinene as an internal standard [22]. To our knowledge, this is the first report of an easy and reliable method for determining arecoline concentrations in dog plasma utilizing LC–MS/MS. Furthermore, we have successfully applied this method to characterize the pharmacokinetics of arecoline hydrobromide tablets in dogs following oral administration at 3 mg/kg.

## 2. Experimental

### 2.1. Chemicals

Arecoline hydrobromide was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) with batch number of 111684-200401 and purity >99%. Internal standard (IS) β-pinene was purchased from TCI Co., Ltd. (Tokyo, Japan). Arecoline hydrobromide tablets were supplied by Lanzhou Institute of Animal Science and Veterinary Pharmaceutics. Methanol (HPLC grade) was purchased from Fisher Chemical (Waltham, USA). All other chemicals were of analytical grade and were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Ningbo, China). Pure water was obtained by purification through a Milli-Q Plus water system (Millipore Corporation, Bedford, MA, USA) before use.

### 2.2. Equipment

The LC–MS/MS equipment (1200-6410A) consisted of a LC system with a binary pump-SL and a triple quadrupole mass spectrometer with electrospray ionization (ESI) (Agilent Technologies, Inc., Santa Clara, CA, USA). Data were recorded, and the system was controlled using MassHunter software (version B.01.04, Agilent Technologies).

### 2.3. Chromatography and mass spectrometry conditions

The separation was carried out at 25 °C on a C18 column (4.6 × 75 mm, 3.5 μm; Agilent Technologies). The mobile phase consisted of methanol:water (the water contained 5 mM ammonium acetate)(10:90, v/v) with a flow rate of 0.40 mL/min. The auto-sampler was conditioned at 4 °C, and the injection volume was 1 μL.

The mass spectrometer was operated in positive ion mode with an ESI interface. Quantitation was performed by multiple reaction monitoring (MRM). In the positive mode, the MS/MS setting parameters were as follows: capillary voltage 4 kV, cone voltage 40 V, source temperature 100 °C, and desolvation temperature 350 °C with a desolvation nitrogen gas flow of 11 L/min and a cone gas flow of 8 L/min. The optimized fragmentation voltages for arecoline and IS were 92 V and 100 V, respectively, and the Delta electron multiplier voltage (EMV) was 200 V. Data were collected in multiple reaction monitoring (MRM) mode using transitions of $m/z$ 156 → 53 (arecoline), with a collision energy of 25 eV, and $m/z$ 137 → 107 (IS), with a collision energy of 20 eV.

### 2.4. Preparation of standard solutions and quality control samples

A standard stock solution of arecoline hydrobromide was prepared by dissolving an accurately weighed, appropriate amount in pure water in a 10-mL volumetric flask to achieve a concentration of 100 μg/mL. A standard stock solution of IS was prepared in anhydrous ethanol and diluted to 0.87 μg/mL. A series of arecoline hydrobromide working standard solutions were prepared by dilutions of the stock solution with pure water to obtain the following concentrations: 2, 5, 10, 50, 200 and 500 ng/mL. All of the standard solutions were stored at 4 °C and brought to room temperature before use. Plasma calibration standards of 2–500 ng/mL were prepared by spiking 10 μL of each standard solution with an aliquot of 100 μL blank dog plasma. Quality control (QC) samples were prepared in the same way at four concentrations of 2 ng/mL (QC-lower limit of quantitation [LLOQ]), 5 ng/mL (QC-low), 50 ng/mL (QC-med), and 500 ng/mL (QC-high). Both the calibration standard and the QC samples were used in the method validation and the pharmacokinetic study.

### 2.5. Sample preparation

Plasma aliquots (100 μL) were spiked with 10 μL of methanol and β-pinene (10 μL of 0.87 μg/mL solution) as an internal standard and mixed (when preparing calibration and QC samples, standard solution was added instead of methanol). Then, 20 μL of $AgNO_3$ solution (0.1 M) and 0.4 mg of NaCl were added followed by vortex mixing for 2 min, and 10 μL of NaOH (1 M) was added followed by vortex mixing for 1 min. Then, 300 μL of *N*-hexane (containing 1% isoamyl alcohol) was added followed by vortex mixing for another 2 min for protein precipitation. Finally, samples were centrifuged at